ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EPIDEMIOLOGY OF BRUCELLOSIS IN CATTLE & ITS SERO PREVELENCE IN ANIMAL HEALTH PROFESSIONALS IN SIDAMA ZONE, SOUTHERN ETHIOPIA

KASSAHUN ASMARE WONDIM

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Epidemiology

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By

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To mom

She did all she could to make me see the light of the day

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ABBREVIATIONS

0- PS	O-chain Lipopolysaccharide
ANRS	Amhara National Regional State
CFT	Complement Fixation Test
DNA	Deoxy Ribos Neuclic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FHD	Full Hemolytic Dose
IAR	Institute of Agricultural Research
ICFTU	International Complement Fixation Test Unit
IFN gamma	Interferon Gamma
IgA	Immunoglobulin A
IgE	" E
IgG	" G
IgM	" M
IL	Interleukin
MHD	Minimum Hemolytic Dose
MOA	Ministry of Agriculture
MRT	Milk Ring Test
NK	Natural killer Cell
OIE	Office International des Epizootics
PAs	Peasant Associations
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
R-LPS	Rough Lipopolysaccharide
S- LPS	Smooth Lipopolysaccharide
SDA	Serum Dextrose Agar
SNNPRS	Southern Nations Nationalities and Peoples Regional State
S-RB51	Strain Rough Brucella 51
SZPEDD	Sidama Zone Planning and Economic Development Department
TAT	Tube Agglutination Test
Th	T-helper cell
TNF-α	Tumor Necrosis Factor α

TSA	Trypticase Soy Agar
VBD	Veronal Buffer Diluents
WHO	World Health Organization

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ABSTRACT

Brucellosis is a widespread infectious disease of livestock and wild life with serious economic and public health impact. Bovine brucellosis causes diminution of much needed human food (milk and meat). Besides, being transmitted to man, it is one of the most important causes of abortion, stillbirth and infertility in cattle. The objectives of this study were, to investigate the current epidemiological picture of brucellosis in cattle in intensive and extensive management systems, identify and describe some of the associated intrinsic and extrinsic risk factors and to see the zoonotic importance of the disease.

The study was conducted from September 2003 to April 2004, in Sidama Zone (Southern Nations Nationalities Peoples Regional state). In the study area, six out of ten *Woredas* were considered for the investigation. Breeding cattle (n=2438) above six months of age were drawn in from both management systems with no history of vaccination. To this effect, a total of 811 Frisian and their crosses from intensive and 1627 indigenous zebu from extensive management system were considered. Furthermore 38 individuals thought to be at risk due to their occupational nature, with particular emphasis given to Veterinarians, Animal health Assistants, Animal Health Technicians, Artificial Insemination Technicians and Meat Inspectors were made part of the study.

Serum samples collected from both animals and humans were screened using Rose Bengal Plate Test (RBPT). Positive reactors were further subjected to Complement Fixation Test to maximize specifity and positive predictive value.

In this study, an over all prevalence of 2.46% in intensive and 1.66% in extensive management systems has been established. Among the risk factors investigated (Age, sex, herd size and management), only age was found to be associated with the infection ($P<0.05$).

Of the total herds investigated, 9.22 % (n=347) of them were confirmed to consist at least one infected animal. The herd level infection rates were 6.72 % (n=223) and 13.70 % (n=124) for intensive and extensive management, respectively. Infected herds have been detected from all *Woredas* except Arroresa. Accordingly, the rate of seroreactor herds observed were, 33.3% (n=6) for Awassa, 15.38 % (n=26) for Yirgalem, 13.15 % (n=38) for Aletawendo, 14.29 % (n=14) for Hagereselam and 18.18 % (n=22) for Arbegona in extensive management. In the

intensive counter part 8% (n=125) for Yirgalem and 5.1% (n=98) for Awassa have been established.

As the observed average herd size was different for either of the management systems, categorizations into small, medium and large herds was done differently. Consequently, in intensive management system, 2.87%, 9.58% and 33.36% were the infection rate for small, medium and large herds, respectively. In the extensive system the result was, 4.81% for small, 50% for medium and 70% for large herds. In both intensive and extensive management systems, the variation between small and large was statistically significant ($P<0.01$).

In intensive urban and periurban dairy, 17.48% of the herds reported at least one of the reproductive disorders (abortion, still birth and fetal membrane retention with or with out any of the disorders).

5.3% of the occupationally risked individuals' sera produced evidence of infection. In the situation where individual animal level infection rate was fond to be low, detection of reactors with this magnitude shows brucellosis being an occupational hazard.

In general the study concluded that, brucellosis is prevalent at low (individual), relatively high (herd) and wide (Geographical) distribution. The infection has involved both management systems (intensive and extensive) and occupationally exposed individuals. The disease among professionally exposed groups needs further validation by including more numbers.

Key Words: Brucellosis, RBPT, CFT, Survey, Zoonoses, Reproductive disorders, serial testing, Intensive management, Extensive management, Occupational groups.

1 INTRODUCTION

Ethiopia is one of such countries with a tradition of livestock keeping. According to the 2003 Central Agricultural Census Commission brief, the livestock population of Ethiopia is estimated at 41,527,142 cattle, 41 million sheep and goats, 1.1 million heads of dromedary (camels), 5,821,297 equines and 52 million chickens. However, this huge potential of wealth is untapped to the lively hood of village farmers and the contribution to national economy at large is minimal. The reason being, preponderance of infectious and parasitic diseases, age-old traditional management system, inferior genetic make up coupled with under nutrition and complicated by malnutrition and absence of well-developed market infrastructure (MOA, 1998).

One of the infectious diseases, which is a major constraint for animal productivity is brucellosis. Animal brucellosis is primarily a disease of ruminants (Cooper, 1991). Brucellosis is a wide spread disease of livestock and human beings resulting in reproductive inefficiency and abortion (Redkar *et al.*, 2001). In dairy production, the disease is a major obstacle to the importation of high yielding breeds and represents a significant constraint to the improvement of milk production through cross-breeding (Mustefa and Nicoletti 1993). The principal manifestations of brucellosis are reproductive failure such as abortion and birth of unthrifty new born in the female and orchitis and epididymitis with frequent sterility in the male (OIE, 2000).

Persistent infection is a characteristic of this intracellular bacterium with shedding in reproductive and mammary secretions (Weidmann, 1991). Infection occurs in cattle of all ages but manifests commonly in sexually mature animals. Congenital infection may also occur in calves born from infected dams. The infection occurs *in utero* and may remain latent in calf throughout its early life and the animal may remain serologically negative until its first parturition (Radostits *et al.*, 1994; Garrin –Bastuji and Verger, 1994).

Brucellosis is also an important zoonosis, threatening public health in many countries of the world (Hamdy *et al.*, 2002; Omer *et al.*, 2002). It is a disease of animals where man is infected as a terminal host. In many countries, it constitutes a large and uncontrolled public health problem. According to the World Health Organization, about half a million cases of human brucellosis occur each year. The prevalence is highest in the Mediterranean countries,

Central and South America, the Middle East and South Asia (Albala, 1995). The major route of human infection in endemic areas is ingestion of unpasteurized milk and its product. In non-endemic areas, occupational exposures through direct contact with infected livestock, or *Brucella* culture represent the major route of transmission via the respiratory tract, conjunctiva, skin abrasion and transfusion of blood (Currier, 1989; Araj and Azzam, 1996).

According to Nicoletti *et al.* (1980) and Staak (1990), brucellosis is perhaps one of the most widespread and economically important diseases in tropical and subtropical regions. The direct loss of meat (because of abortion, infertility, and weight loss) in infected herds of cattle was estimated to be 15% and for milk (reduction in milk production) 20% per infected cow.

In Ethiopia the growing tendency of intensification in dairy, especially in urban and periurban areas has instituted the practice of improving the blood level of the dairy herds. It has a vision of increasing local animals' productivity. However, the limited number of surveys done by few individuals (Molla, 1989; Wondimu, 1989; Zewdu, 1989; Asfaw *et al.*, 1998 and Bekele *et al.*, 2000) showed high prevalence of bovine brucellosis in exotic and crossbred animals compared to local zebu cattle.

As the disease is hardly spectacular in its chronic stage and despite the losses and yield decrease it causes often goes undetected (in contrast to Rinderpest). Its negative effect on profitability of cattle production is extremely underestimated particularly in tropical areas (Weidmann, 1991).

The introduction of SDDP (Smallholder Dairy Development Project) in the year 1995 and SDP (Sidama Development Project) there after has boosted the pre-existing small urban and periurban dairy activity. This was through adoption of improved methods such as fodder production, management facility and supply of cross breeds on credit in kind basis (SZPEDD, 2001). The increased livestock development activity combined with rapid population pressure in urban and periurban areas, has opened new market and favored the sector positively.

In urban areas of Sidama Zone, due to the rising demand of milk and attractive market, dairying is actively growing and has become a sideline business for most civil servants. To this effect, the level of intensification and introduction of exotic animals has been observed to increase in the last two decades. However, an organized surveillance on brucellosis has not been done. Similarly, no available information on the zoonotic aspect of the disease does

exist. Therefore, such limited information constituted the rationale for this study, which is supposed to describe the current epidemiological status of the disease in cattle and its seroprevalence in occupationally exposed groups in Sidama Zone, Southern Ethiopia.

Objectives

1To state the prevalence of brucellosis in cattle, both in intensive and extensive management system

2 To investigate the status of brucellosis in occupationally exposed groups

3 To investigate some of the host, environment and management risk factors

2 LITERATURE REVIEW

2.1 Etiology

Brucella species are gram-negative, often appear cocco- bacillary, non-spore forming, non-motile partially acid- fast (Quinn *et al.*, 1994). On the basis of 16S rRNA gene sequence comparisons, the genus *Brucella* is grouped in alpa-2 subdivision of class *Protobacteria.* The genus is divided based on cultural, metabolic, antigenic properties and host specificity into six species: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (Redkar *et al.,* 2001).

The principal cause of bovine brucellosis (Contagious abortion), has seven biotypes and a number of strain variants (Garrin- Bastuji and Verger, 1994). About 85% of infections are from biotype 1. There are no proven differences in the pathogenecity or antigenicity among field strain biotypes (Nicoletti, 1980). Biovar 1 is the type most frequently isolated from cattle worldwide. Biovars 3 and 6 are common in Africa and some Asian countries. Biovars 2 and 4 in addition to biovar one found in North and South America where as biovars 5 and 7 are rare, but have been locally important in some countries in the past, e.g. Britain and Germany (OIE, 1996).

Brucella melitensis usually infects goats and sheep and *Brucella suis* is pathogenic to pigs (Redkar *et al.*, 2001). There are three biovars of *Brucella melitensis* which have differing geographical distribution but no difference in pathogenecity or animal species affected (Radostits *et al.*, 1994). *Brucella ovis* is an important cause of epididymitis and orchitis in sheep. It is not recognized as a cause of natural infection in goats, except experimental (Smith and Sherman, 1996).

To date human infection by *Brucella* organism has been caused by four species: *Brucella melitensis, Brucella abortus, Brucella suis* and *Brucella canis* (Araj and Azam, 1996; Madkour and Kasper, 2001).

2.2 Epidemiology

2.2.1 Occurrence

Despite advances made in diagnosis and therapy, brucellosis is still widespread and prevalent in many developing countries (Mahajan and Kulshreshtha, 1991). In 1986, bovine brucellosis was recorded in 120 of 175 countries. Thirty-three countries did not record the disease and the data were not available from the remaining (Radostits *et al.,* 1994). Host preference is exhibited by the different *Brucella* species (Quine *et al.*, 1994). However; a broad host range has been demonstrated for some species. Survival time outside the host is variable and depends on the temperature and moisture. Cold weather extends survival time (Walker, 1999).

Strictly speaking, the different species of *Brucella* are not highly host specific. Thus, their evolutionary adaptability and inter-host transmission continues to change (Verma *et al.*, 2000). These days, *Brucella melitensis* in cattle has emerged as an important problem in some Southern European countries, Israel, Kuwait, and Saudi Arabia. A related problem has been noted in some South American countries, particularly Brazil and Colombia, where *Brucella suis* biovar 1 has become established in cattle (Corbel *et al.*, 1997). Isolation and identification fail in surprisingly high proportion or cases. This is believed to be due to the fact that many of fastidious Brucella are elusive and their presence is fleeting some are extremely delicate and survive poorly in rapidly decaying carcasses (Verma *et al* ., 2000).

2.2.2 Transmission

Transmission is by direct or indirect contact with infective excretions. The route of infection is often by ingestion. Less commonly infection may occur in *utero,* via conjunctiva or by inhalation. Animals may ingest contaminated feed and water, or lick contaminated genitals of other animals. Venereal transmission by infected bulls to susceptible cows may rarely occur (Seifert, 1996; Weidmann, 1991).

Infection of the accessory sex glands of male allows dissemination of organisms through the semen. Insects may have a minor role in the transmission and maintenance of infection in the herd. Face flies have been shown to take up and excrete *Brucella* in feces (Walker, 1999). Herding plays an important role in epidemiology of brucellosis. This is due to crowding and

contact, which increase disease transmission and sustain endemicity (Cooper, 1991; Omer *et al.*, 2000a).

2.2.3 Risk factors

2.2.3.1 Management

From practical point of view, the factors influencing the transmission of brucellosis in any given geographical region can be classified into two fundamental categories (Radostits *et al.*, 1994).

I. Factors influencing inter-herd transmission

This includes purchase of infected replacement animals, which are in turn influenced by the frequency of purchase, source of purchase and brucellosis test history of purchased animals. The proximity of infected herds to clean herds is an important risk factor. Cattle contact at fence lines, sharing of pastures and strays of infected animal into clean herds are common methods by which transmission occurs to adjacent herds.

II Factors associated with maintenance and spread within the herd

This includes unvaccinated animals within the herd, herd size, population density, and method of housing and use of maternity pen. Large herd sizes are often, maintained by the purchase of replacement of cattle which may be infected. It is also more difficult to manage large herds, which may lead to managerial mistakes that allow the disease to spread. There is a positive association between population density (number of cattle to land area) and disease prevalence, which is attributed to increased contact between susceptible and infected animals (Omer *et al.*, 2000b). The use of maternity pen at calving is associated with the decrease in the prevalence of infection presumably due to decreasing the exposure of infected and susceptible animals (Nicoletti, 1980).

2.2.3.2 Host factor

Brucellosis infects a variety of domestic and wild animals and man causing incapacitating disease (Hamdy *et al.*, 2002). It is essentially a disease of sexually mature animal, the predilection sites being the reproductive tract of males and females especially the pregnant uterus. Apparently normal but infected neonates can be born, but the infection is of limited duration in these animals. Females usually abort only once, after which a degree of immunity

develops, and the animals remain infected and large number of *Brucellae* can be excreted in the fetal fluids at subsequent parturitions (Quinn *et al.*, 1994).

2.2.3.3 Pathogen factor

The inability of leukocytes to kill virulent *Brucella abortus* effectively at the primary site of infection is a key factor in the dissemination to the regional lymph nodes and other sites. The organisms are capable of surviving and multiplying inside macrophages by inhibiting phagolysosome fusion. *Brucella* resist intracellular phagocytic killing by suppression of myelo-peroxide- hydrogen peroxide-halid system and production of super-oxide dismutase (Madkour and Kasper, 2001). Stress proteins have been demonstrated in *Brucella* and could be a factor for intracellular survival in the host. These proteins are thought to play a role in protecting organisms from hydrolytic enzymes, oxygen radicals and myeloperoxidase killing system in the phagolysosome. The lipopolysaccharide of *Brucella* is directly associated with virulence and is thought to play a role in enhancing intracellular survival (Walker, 1999).

2.3 Bovine brucellosis in some African countries

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange Free State of South Africa in the year 1915 (Chukuwu, 1985). However, the surveillance and control of brucellosis in sub-Saharan Africa is rarely implemented outside South Africa (McDermot *et al.*, 2002). Most surveys have been conducted on selected herds or areas and is there fore very difficult to asses national prevalence's for each country. Yet, it is apparent that bovine brucellosis is wide spread in Africa and in most countries, high prevalence is recorded (Madson, 1989).

A report of 13.2% - 46% made from Zimbabwe (Kagumba and Nandoka, 1978), 9.5% in southern part of Somalia (Wernery *et al.*, 1979), 35.1% in *Ankole* breeds of Ruwanda (Akakpo *et a*l., 1994), 5% in zebus of Ouganda (Kagumba and Nandoka, 1978) and 6.5% in *Dinka* and 22.5% in *Felata* cattle of Southern Sudan (Hellman *et al.*, 1985) shows the overview in some East African countries (Annex-4).

2.4 Diagnosis

Diagnosis of brucellosis is the cornerstone of any control program and is based on bacteriological, immunological findings (Hamdy *et al.*, 2002) and molecular biology (DNA probe) (Redkar *et al.*, 2001).

2.4.1 Bacteriology

Bacteriological method is the most dependable as the identification of the pathogen itself is unequivocal (Weidmann, 1991). Smears of placental cotyledon, vaginal discharge and fetal lung, liver and abomasal contents should be fixed with heat or ethanol and stained by Modified Ziehl-Nelson (Stamp's), Kosters', Gram or Macchiavello methods, or with a fluorochrome or peroxidase-labeled antibody conjugate.

The presence of large aggregates of intracellular weakly acid-fast organisms of *Brucella* morphology or immunospecifically stained organisms is presumptive evidence of brucellosis. Care must be taken in the interpretation of results as other infectious agents may have similar morphology (e.g. *Coxiella burneti, Chlamydia psittaci*).

Culture of placental cotyledon, vaginal discharge, fetal tissues or abdominal contents, or hygroma fluid should be made on solid media of appropriate selectivity. There are many varieties of suitable media, such as serum dextrose agar (SDA), trypticase soy agar (TSA) and blood agar supplemented with bacitracin (25 mg/ml= 25,000 u), vancomycin (20mg/ml). SDA with the addition of these antibacterials is also known as Farrell's modified SDA. The addition of extra serum to the medium enhances the growth of some *Brucella* strains (OIE, 2000).

2.4.2 Serological Diagnosis

The diagnosis of brucellosis is best established when the causative organism is isolated from blood or other body fluids in suspected cases (Albala, 1995). However, achievement of an infallible diagnosis of brucellosis is a tedious process, since isolation is influenced by a number of factors, such as high fastidious growth requirement, a lesser number of viable organisms in the sample, delay in transportation (leading to putrefaction), and earlier treatment with chemotherapeutics. In addition, a prolonged incubation period may lead to

failure in its isolation (Konrad, 1981; Verma *et al.*, 2000). Thus, there has been an increasing reliance on the use of serological tests as the means of indirect diagnosis (Staak, 1990; Hamdy *et al.*, 2002).

The S-LPS more specifically O-chain plays a central role in the serological diagnosis of brucellosis. It is an important antigen which is able to induce antibody response in most animals exposed to smooth *Brucella* organisms and most diagnostic serological tests are based on the detection of antibodies to the O-chain (Schuring, 1998). In the diagnostic assay, the antibodies to be assessed must be capable of secondary function, such as agglutination, activation of complement or precipitation to be measured (Nielsson *et al.*, 1996).

The buffered *Brucella* antigen tests e.g. Rose Bengal test, buffered plate agglutination tests, enzyme-linked immunosorbent assay, or florescence polarization assays are suitable tests for screening herds and individual animals. The reactivity of samples that are positive in screening tests should be retested using established confirmatory tests such as complement fixation test or enzyme linked immunosorbent assay. The serum agglutination test is inferior to other tests in specificity and sensitivity and is not recommended if other procedures are available (OIE, 1996).

The indirect enzyme linked immunosorbent assay or milk ring test performed on bulk milk samples are effective for screening and monitoring dairy cattle for brucellosis, but the milk ring test is less reliable in large herds and less sensitive with *Brucella melitensis*. Another immunological test is the brucellin skin test, which can be used as screening or as confirmatory herd test in the unvaccinated herds. A purified standardized preparation is available (OIE, 2000).

2.4.3 Animal Inoculation

Animal inoculation given either subcutaneously or through abraded skin in guinea pigs, or preferably intravenously in mice may some time provide the only means of detecting the presence of *Brucella*. Especially, when samples are heavily contaminated or likely to contain a low number of organisms. The spleen of mice are cultured 7 days after inoculation and for guinea pigs, the serum sample is subjected to specific tests 3 and 6 weeks after inoculation, then the spleens are cultured (OIE,1996).

2.4.4 Molecular Biology

Many current serological tests have proved to be either too sensitive, giving false positive results, or too specific, giving false negative results. In addition, the presence of antibodies doesn't always mean an active case of brucellosis due to post-vaccinal immune response and cross reaction with other gram negative bacteria e.g. *Yersinia enterocolitica* (Hamdy *et al.*, 2002).

PCR with random or selected primers gives promising results, but standardization and further evaluation are needed, especially for chronic disease. Similarly, antigen detection methods are potentially useful but have not been validated. Combinations of these with PCR, such as immuno-PCR, have considerable potential but require evaluation (Corbel, 1997).

2.5 Immunity

Brucella are facultative intracellular bacteria that survive and replicate in both phagocytic and non phagocytic cells. Phagocytes play a key role in initiating T-cell responses by processing and presenting antigens (Tizard, 1996). T-cells play a major role in the acquired specific resistance to intracellular bacteria determining the resolution of infection. Protective cell mediated immunity to *Brucella abortus* in mice has been demonstrated by passive transfer assays with immune T- cell enriched spleen cells. The combined transfer of immune serum and cells has given better protection than that provided by serum or cells alone given prior to challenge (WHO, 1997).

2.5.1 Humoral Immune Response

Brucella abortus, *Brucella melitensis*, *Brucella suis* and *Brucella neotomae* species may occur as either smooth or rough strains expressing smooth lipopolysaccharide (S-LPS) or rough lipopolysaccharide (R-LPS) as the major surface antigens. *Brucella ovis* and *B. canis* are two naturally rough species expressing R-LPS as the major surface antigen. S-LPS (smooth lipopolysaccharides), precisely O–PS (O-chain polysaccharide) specific antibodies play a major role in protective immunity against brucellosis caused by smooth *Brucella* (WHO, 1997).

The immunoglobulin isotopes present in bovine serum are IgG_1, IgG_2, IgM and IgA. IgA concentration in bovine serum is usually very low and the role of this isotope in the various serological tests has not been clearly defined. Secretary IgA in milk does play an important role in the milk ring test. IgM also participates in this reaction (Sutra *et al.*, 1988). In cattle naturally infected with *B. abortus,* there is a subsequent rise in both IgM & IgG antibodies. However, the IgM titer declines and later the predominant immunoglobulin present is IgG. In some cases of chronic brucellosis, the animal can have high level of IgG1 that agglutinate poorly itself and can also mask the normally efficient agglutinating properties of IgM antibodies that may be present (Quinn *et al.*, 1994).

The first isotope produced after an initial heavy infection or S-19 immunization is IgM. This can usually be detected in the first or second week following the initial antigenic stimulus, but is soon followed by IgG antibody (Tizard, 1996).

2.5.2 Cellular Immune Response

Macrophage that ingest live *Brucella* secret different set of cytokines (Tizard, 1996). The derived cytokines such as IL_1, IL_{12} and TNF–alpha contribute to the control of early *Brucella* infection; $IL\text{-}_{12}$ by stimulating NK cells and T-cells to produce IFN– gamma and TNF–alpha via an IFN–gamma independent pathway, probably by recruiting phagocytes to the site of infection, activating macrophages and promoting granuloma formation (Madkar and Kasper, 2001). IFN–gamma is one of the most important T–cell stimulated cytokines in resistance to *Brucella abortus* infection. It is potent activator of macrophages and monocytes and regulates their metabolic activities to produce oxidative metabolites and other microbicidal molecules (WHO, 1997).

Indeed, IFN–gamma has been reported to reduce *Brucella* growth in macrophages although activation with IFN- gamma alone does not result in total elimination of intracellular *Brucella*. Factors working with IFN–gamma for elimination of intracellular *Brucella* include iron, TNF–alpha and nitric oxide production plays a role in killing the bacteria. In contrast, IL-10 and IL-4 contribute negatively to resistance of infection by down regulating both Macrophage effecter function and the production of IFN- gamma.

In general, the antibodies against O-PS and Th_1 cellular response (IL_1–associated cytokines / IFN–gamma) are required for optimal protection against smooth *Brucella* (WHO, 1997).

2.6 Importance of bovine brucellosis

2.6.1 Economic Importance

It is ideal to have every pregnant cow to go term and have a healthy calf. However, some loses due to abortion are expected and the maximum loss of 3% is acceptable. The economic loss of brucellosis is mainly due to abortion. This depends on direct costs from value of fetuses lost. The indirect cost include those associated with establishing the diagnosis, re-breeding cows that aborted, possible loss of milk and replacement cost of culling (Peter, 2000).

Losses in animal production due to this disease can be of major importance, primarily because of decreased milk production by aborting cows (Rana *et al.*, 1985). The common sequel of infertility increases the period between lactations and in infected herds, the average intercalving period may be prolonged by several months. In addition to the loss of milk production, there is loss of calves and interference with the breeding program. This is of greatest importance in beef herds where the calves represent the sole source of income. A high incidence of temporary and permanent infertility results in heavy culling of valuable cows and some deaths occur as a result of acute metritis following retention of fetal membrane (Radostitis *et al.*, 1994; Weidmann, 1991).

2.6.2 Zoonotic Importance

Brucellosis is considered by the Food and Agricultural Organization (FAO), the World Health Organization (WHO) and the "Office International des Epizootis" (OIE) as one of the most wide spread zoonoses in the world (Schelling *et al.*, 2003). The main sources of human infection are domesticated food animals: cattle, sheep, goats, and swine and in some countries buffalo, yak and camels. Human beings are susceptible to infection with *Brucella melitensis, Brucella suis, Brucella abortus* and *Brucella canis* but infections caused by *Brucella melitensis* are known to cause more severe clinical and pathological effect (Omer *et al.*, 2002). Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *Brucella abortus* is still the most widespread form and responsible for most world wide morbidity, particularly in developing countries(Corbel, 1997). And its infection can also be severe and life threatening, especially when resistance is very low due to preexisting disease or malnutrition (Omer *et al.*, 2002).

Although progress in the control and local eradication of brucellosis in several parts of the world has been achieved, brucellosis remains a major public health hazard (Garrin-Bastuji and Verger, 1994). Human beings are accidental and almost dead end host of *Brucella* infection. The disease is primarily an occupational risk and occurs mainly in exposed professionals such as veterinarians, farmers, laboratory technicians, abattoir workers and others working with animals and their products. Animal health workers are infected most commonly from exposure to placental tissue, fetal fluid, aborted fetus and newborn calves. In some cases, infection has resulted in advertent self-inoculation or syringe splash to the face or eyes from *Brucella abortus* strain 19 vaccine. Others at risk include, people who consume unpasteurized dairy products (Russel, 1989).

In human beings, the disease is characterized by a multitude of somatic complaints, including fever, sweating, anorexia, fatigue, malaise, weight loss and depression. Localized complications may involve the cardiovascular, gastrointestinal, genitourinary, hepatobiliary, osteoarticular, pulmonary and nervous systems. Without adequate and prompt antibiotic treatment, some patients develop a 'chronic' brucellosis syndrome with many features of 'chronic fatigue' syndrome (WHO, 1997).

2.6.2.1 Occurrence of human brucellosis

The true incidence of human brucellosis is unknown. Reported incidence in endemic-disease areas varies widely, from <0.01 to >200 per 100,000 population. While some areas, such as Peru, Kuwait, and parts of Saudi Arabia, have a very high incidence of acute infections, the low incidence reported in other known brucellosis-endemic areas may reflect low levels of surveillance and reporting (Corbel, 1997).

Human Brucellosis in Africa is widely documented. Prevalence figures as high as 30% have been reported from Kenya, while other African countries Tanzania, Uganda, Somalia and Sudan reported prevalence rates with in the range of 1-25% (Madson, 1986). In Eritrea, a prevalence rate of 7.1% among dairy workers, 4.5% veterinary Personnel and 3% were reported in pastoral community (Omer *et al.*, 2002).

In nutshell, the prevalence of brucellosis among human population is largely influenced by the prevalence of the disease among domestic animals. Local tradition regarding the proximity of animal housing, human habitation and the consumption and processing of milk

products have immense role. Furthermore, the type of *Brucella* species present in the region, the type of domestic animals, climatic condition and standard of personal and environmental hygiene complementing the disease occurrence (Omer *et al.*, 2002).

2.7 Prevention and control

According to Walker (1999), approaches in control and prevention of brucellosis depend on the animal species involved, *Brucella* species and management practice and on availability and efficacy of vaccines. Approaches used to control brucellosis include:

- Immunization
- Testing and removal of infected animals in conjunction with immunization program.
- Management practice

2.7.1 Immunization

As a rule, induction of an effective long-lasting, protective immune response, to facultative intracellular organisms require the use of live vaccine or in some cases the use of multiple injection of appropriate protective antigen the presence of adjuvant (WHO, 1987).

Immunization reduces the number of abortions and thereby reduces potential for exposure. By itself, immunization will not result in eradication of the infection in a herd. Immunization should be considered as a means of controlling the level of disease only (Seifert, 1996; Walker, 1999).

2.7.1.1 Live attenuated vaccines

2.7.1.1.1 Strain- 19

As a broad generalization, living bacteria are much more capable of activating macrophages than are inactivated organisms , and macrophage that ingest live *Brucella* secret a different set of cytokines than macrophages ingest killed *Brucella* (Tizard,1996).

S -19 is the most commonly used live attenuated vaccine for protecting cattle against brucellosis (Uzal *et al.*, 2000). The vaccine has been applied for a long time world wide for

immunoprofilaxis (Seifert, 1996). It is normally given to female calves aged between 3 and 6 months as a single subcutaneous dose of 5-8 x 10^{10} viable organisms. Reduced dose from $3x10^8$ to $3x10^9$ organisms can be given in beef or dairy cattle aged between 4-12 months, but 5-10% will develop persistent antibody titer. Alternatively, it can be administered to cattle of any age as two doses of $5x10^9$ viable organisms given by conjunctival route (OIE, 2000). Although the vaccine may not completely protect infection, it has been successfully used to prevent abortion (Tizard, 1996).

Strain- 19 is of low virulence for cattle, however; subcutaneous injection of pregnant cattle may result in abortion but incidence ranges from less than 1% to 2.5% under field conditions (Schuring, 1998). Furthermore, it cannot protect brucellosis in cattle caused by *Brucella melitensis* (Corbel *et al.*, 1997).

2.7.1.1.2 *Brucella melitensis* Rev.1

Rev.1 vaccine is an attenuated live *Brucella melitensis* strain originated from a virulent *Brucella melitensis* isolate, which become dependant on streptomycin for its growth but lost this characteristic upon further culture. It gives protection to sheep and goats against infection with *Brucella melitensis* and to rams against *Brucella ovis*. This vaccine is attenuated when compared to field strains but retains some virulence. Depending on the dose administered during pregnancy, abortion will occur frequently. The use of Rev-1 in cattle has been investigated and results indicate that Rev-1 protects well and better than strain-19 (Schuring, 1998).

It is given to lambs and kids between 3 and 6 months as a single subcutaneous dose of 10^9 viable organisms. The use of this vaccine administered by the conjunctival route with a standard dose or $1-5x10^8$ viable organisms produces similar protection without persistant antibody titer (OIE, 1996).

2.7.1.1.3 S-RB 51

B. abortus RB 51 is a new vaccine recently developed ,which has similar efficacy to S-19 in protecting cattle against brucellosis. It is an attenuated rough organism which essentially lacks the O- chain of LPS (Uzal *et al.*, 1997). This vaccine can be administered a number of times with out inducing antibodies reactive to conventional serological tests (WHO, 1997). The

vaccine has replaced *B .abortus* S-19 in mony countries and has been approved officially (Walker, 1999). The dose rate varies from 1-3.4x10^{10} viable organisms for calves 4-12 months to adults receive 1x10^{9} viable strains 51. The vaccine is less abortigenic compared to S-19 and Rev.1 (OIE, 2000).

2.7.1.2 Inactivated vaccines

2.7.1.2.1 Strain 45/20

In order to overcome the lack of stability of live strain 45/20, it is used as bacterin incorporated with adjutants; usually based on water and oil emulsion (Schuring, 1998). Two doses are given 6-12 weeks apart, followed by annual booster to maintain immunity (OIE, 1996). This vaccine can also be used for anaminestic diagnosis in latent carriers (Weidmann, 1991). The other advantages of strain 45/20 are safe with respect to residual virulence and relatively easy to store (Tizard, 1996).

2.7.1.2.2 H-38

It is produced from attenuated *Brucella melitensis* strain containing aluminum hydroxide as adjutants. H-38 is mainly for small ruminants (Seifert, 1996). The vaccine provides good protection after a single application by producing higher level of all immunoglobulin classes (Weidmann, 1991).

2.7.2 Immunization followed by test and slaughter

Control of bovine brucellosis routinely employs a combination of vaccination of females and test and slaughter program. Cattle are vaccinated at a young age and tested by immunodiagnostic tests when they reach sexual maturity. Vaccination with S-19 is approximately 70% effective on an individual basis, but more effective when evaluated on a herd basis. Any animal identified as infected is culled from the herd and slaughtered (Walker, 1999).

Immunization usually entails reducing the incidence of the disease and demands hygienic steps. When the rate of infection is reduced to acceptable level (1-2%) test and slaughter can

be launched. This step is rigorously carried out until two successive precise herd tests conducted six weeks apart yield negative result (Weidmann, 1991).

2.7.3 Management practice

From the epidemiology of the disease, there are important steps to be implemented at an early stage. These include the isolation of calving animals in separate calving pens, which are subsequently disinfected, the burning and burial of placentas, aborted fetus, etc., testing and quarantine of newly arrived animals and regular examination of the herd. If one owns a disease free herd, contact with neighboring herds must be prevented (Nicoletti, 1980; Garrin-Bastuji and Verger, 1994).

2.7.4 Prevention in human beings

Prevention of brucellosis in humans still depends on the eradication or control of the disease in animal hosts, the exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods. Vaccination now has only a small role in the prevention of human disease (Corbel, 1997).

2.8 Epidemiology of bovine brucellosis in Ethiopia

2.8.1 Prevalence

The limited number of surveys done on the prevalence of bovine brucellosis both in extensive and intensive livestock management has already indicated how important the disease is in different parts of the country.

The endemicity of bovine brucellosis in Ethiopia has been gradually established over the last two decades by various researchers; 8.86% in Arsi (Molla, 1989), 15.6% in Sidamo (Zewdu, 1989), 22% in Chafa state farm, Wello (Sintaro, 1994), 8.11% in and around Addis Ababa (Asfaw *et al.*, 1998) Bekele *et al.* (2000) had reported as high 19%in Abernossa ranch.

Rashid (1993) reported a prevalence rate of 38.7% (57/147) at Bako Research Center, Western Ethiopia. Recently, Kebede (2000) and Mekonnin (2001) have done a very extensive survey in Amara National Regional State and reported a prevalence rate of 1.8% and 8.3% in eastern and western part of Amara Regional State, respectively (Annex-5). The disease thus appears to be widespread in both indigenous and exotic crosses of cattle in the country.

3 MATERIALS AND METHODS

3.1 Study area

The study was carried out in Sidama Zone of Southern Nations Nationalities and Peoples Region. The zone is located in northern part of SNNPRG, with its capital at Awassa, which lies about 275 km south of Addis Ababa (Annex 6). Geographically the zone lies between 4^0 27' and 8^0 30' N latitude and 34^0 21' and 39^0 11' E longitude (SZPEDD, 2001).

Like most parts of Ethiopia, the relief configuration of Sidama ranges from very high mountains to lowland plains, where the altitude varies between 1001 to 3200 m.a.s.l. The mean annual temperature is 20.1^0c, with monthly means ranging 18.1^0c in November and December to 20.4^0c in March; however, the diurnal variation can be very wide reaching 30^0c in the dry season. The mean annual rainfall is 960mm, with the rainfall having bimodal regime i.e., two rainy seasons in a year. The small rainy season *Belg* starts from February to May and the big rain *Meher* starts from mid June to October. In many parts of Ethiopia, the *Belg* merely serves for preparation of land in readiness for cultivation during *Meher*. In the study area, however the *Belg* is heavy enough in most years for farmers to start early cropping. Maize, *enset* (*Enset ventricosum*), cash crops like *chat* (*Cata edulis*) and coffee are planted during March to May. Dry savanna, bush, dry mountain forest, and Agro forestry are the four types of vegetation which characterize Sidamas green coverage (SZPEDD, 2001).

3.2 Livestock population and economic activity

According to Sidama Zone Agricultural Department statistical abstract (2000), the total livestock population of Sidama Zone is estimated to constitute 653,100 cattle, 316,620 goats, 404,130 sheep and 194,530 equines. As elsewhere in rural Ethiopia, the economic life of the people in the zone is mostly dependant on mixed farming in that 93% of the population is engaged in agriculture.

Livestock production occupies an enormous share in farm economy. The high and mid Landers are sedentary while transhumance is the style for lowlanders. The most important food crops produced are *enset*, maize, wheat, barley, *teff*, pulses and coffee as an important cash crop (SZPEDD, 2001).

3.3 Study design

A seroepidemiological study was carried out in districts of Sidama Zone from September 2003 to April 2004. The study subjects include cattle and animal health professionals such as veterinarians, animal health assistants, animal health technicians, AI technicians and meat Inspectors. Blood samples were collected from breeding animals above six months of age and specified professionals. Screening of serum was done using Rose Bengal Plate Test (RBPT). Sera testing positive, were tested further by Complement Fixation Test (CFT). This serial testing maximizes specificity and predictive value of positive test result. The questionnaires survey conducted to intensive farm owners, professionals and paraprofessionals were used to evaluate some of the associated risk factors.

A cross sectional epidemiological study was carried out to determine prevalence of bovine brucellosis and the role of risk factors like age, sex, herd size, under intensive and extensive management systems. To this effect herds of cattle was categorized in the following groups based on the management systems.

I-Urban and periurban dairy involving exotic (Friesian) and crossbreeds in intensive management. Herds were characterized in to small (1-3 animals), Medium (4 -9 animals) and large (>9 animals) herds.

I I - Cattle of local breeds (zebu) in extensive management characterized in to small (1-15animals), Medium (16-30 animals) and large (>30animals) herds.

3.4 Sampling methods

3.4.1 Study animals

Cattle a- Urban and periurban dairy involving exotic (Friesian and cross bred) in intensive management

b- Cattle of local breeds (zebu) rose in extensive management.

In the above classification, all animals above six months of age were considered. The sampling frame of herds for Yirgalem and Awassa dairy herds were prepared with the help of respective woredas animal and fishery resource development desk. On the other hand, the sampling frame for extensively managed herds was prepared based on list of PAs for the zone. In selected PAs, cattle sampled were not limited to those villages with in the PAs, rather; cattle sharing the communally owned grazing land were also involved.

3.4.2 Sample size

A two-stage cluster sampling technique has been used to calculate the actual sample size having the following parameters predetermined. CL=95%, Desired level of precision 5%, Expected total clusters prevalence 15.8 % (Zewdu, 1989) and in between cluster variance 0. 000625.

Average individually owned herd size was determined to be ten (10) animals for extensive and five (5) animals for intensively managed ones. The in-between cluster variance was determined by guessing the standard deviation (i.e. the average difference expected between individual cluster prevalence and the overall mean cluster prevalence). Squaring the standard deviation results in the variance component between clusters (Thrusfield, 1995).

Then using the formula $g = \frac{1.96^2(nVc + Pexp(1-Pexp)}{nd^2}$

Where n= herd size

vc = in between cluster variance

d = desired level of precision

pexp = expected prevalence

g = number of clusters needed

A total of 64 clusters (42 herds from intensive and 22 herds from extensive management) were to be picked randomly from of the prepared sampling frame.

In order to get the total sample size, $Ts = \frac{1.96^2 * g * Pexp(1-Pexp)}{gd^2 - 1.96^2 Vc}$

The above formula has been used for both cases. However, the samples taken were nearly six fold of the formula output. For occupationally risky groups, an attempt was made to communicate all the willing individuals engaged in the profession and 38 (66.7%) of them were sampled.

A total of **1627** indigenous animals found in **124** herds (Pas) kept under extensive management and **811** exotic and their crosses in **223** herds from intensively managed once

were sampled. These animals were taken from six *Woredas* and all animals sampled were above six months of age. To asses the occurrences of the disease in human sample was taken from occupationally exposed groups.

3.5 Materials needed

A RBPT (Rose Bengal Plate Test)

1. RBPT Antigen
2 .Negative & positive control sera
3. Test serum
4. Plastic applicator
5.Enamel plate or glass slide
6. Rocking machine and centrifuge

B. CFT (Complement Fixation Test)

1.Microwel plates (U-shaped), multi channel and single channel pipettes, pipette tips
2. Flasks and measuring cylinders
3. Beam (digital) balance
4. Incubator, water bath, deep freezer, centrifuge
5. Veronal buffer and Alsever's solution
6. Complement, hemolisin (amboceptor's), control sera and sheep RBC
7. CFT Antigen

3.6 Serological Tests

3.6.1 Rose Bengal Plate Test

Serum of 30µl was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately equal to 2 cm in diameter. The mixture was rocked, gently for four minutes at ambient temperature and then observed for agglutination. Any visible reaction was graded positive and otherwise negative (OIE, 2000).

Procedure

1. Holding the antigen dispenser upright, 30µl of RBPT antigen obtained from pourquier instiut 326, rue, de la galera 34097 Montpelier cedexs were placed on each circle on the plate.
2. Thirty micro liter of test serum placed along side, but not on the antigen
3. With plastic applicator stick, the antigen and serum was mixed thoroughly
4. The plate was rocked on rocking machine and mixed for four minutes

3.6.2 Complement Fixation Test (CFT)

The test was done at National Veterinary Institute and preparation of the reagents was made according to protocols of the procedure. Antigen, control sera and compliment were obtained from the BgVV, Berlin, Germany.

I-Preparation of sheep red blood cells for the hemolytic system:

Ten (10) ml of sheep red blood cells in Alsever's solution was centrifuged at 2500rpm for 5 minutes. The supernatant was discarded and replaced by veronal buffer diluent (VBD). The sheep red blood cell was resuspended in diluents completely. This procedure was repeated four times. Before discarding the supernatant after the last washing, the volume of the packed cell was measured. The volume of the packed cell was measured by placing an identical tube next to the blood containing tube filled up to the level of blood by a measured amount of water. By addition of calculated amount of VBD, 2% sheep red blood cell suspension was prepared.

II-Amboceptor's titration:

1. Prepare1:500 amboceptor and dilute serially to 1:8000
   - **a**/ prepare 5 test tubes
   - **b**/ Add 1 ml of VBD to test tubes 2-5
   - **c**/ Mix10µl amboceptor with 4990µl VBD in the first tube
   - **d**/ Transfer 1 ml from the first to the second up to the last tube and discard 1 ml
2. Prepare 1:750 amboceptor and dilute serially up to 1:12000
   - **a**/ Prepare 5 test tubes and add 1 ml of VBD to test tubes 2-5
   - **b**/ Mix 10µl amboceptor with 7490µl in the first tube
   - **c**/ Transfer 1 ml from first tube to the second up to fifth tube and discard 1 ml. Put the tubes in order of ascending dilution
3. Transfer 0.5ml from each of these test tubes to a second set of 10 tubes. Start with the 1:12000dilution

4. Add 1.ml of VBD to each of the test tubes

5. Add 0.5ml of 2% SRBC to each of the test tubes and shake well

6. Leave on the bench for 10 minutes

7. Add 1 ml of complement at working dilution

8. Incubate tubes for 30 minutes in a water bath at 37^0C

9. Read and record the last tube showing complete hemolysis (MHD)

*The working dilution of amboceptor is four times the MHD

III-Evaluation of complement.

1. Freeze dried complement was reconstituted according to its instructions

2. A 1:100 complement was prepared

3. Complement was added into the 9 wells increasing by 5µl every time, starting with 10µl

4. Diluent was added into the 9 wells in decreasing amount by 5µl, starting with 40 µl

5. 25 µl of diluent was added into the wells with the Cornwall syringe

6. The plate was placed in water bath at 37^0c for 1hr

7. 25 µl of 2% sheep red blood cells was added in all wells

8. 25µl of amboceptor at working dilution 1:1000 was added in all wells

9. The tubes were properly mixed and put again in the water bath of 37^0c for another 30 minutes.

10. The test was read by recording the minimum hemolytic dose of complement (MHD), which was represented by the first well showing complement hemolysis. The next well contains the full hemolytic dose (FHD).

The complement dilution=2FHD/Dilution of complement

IV-Titration of antigen

Micro titer plate I

1. 25µl of VBD was added in all wells

2. 25µl pre-diluted antigen was added to all wells of row A

3. By serial doubling (two fold) dilution 25µl of antigen was transferred from row A to B, and again from row B to C, etc. until row G by multichannel pipette; 25µl mixture was discarded from row G (row H had only the diluents).

Micro titer plate II

1. 50µl of VBD was added to all wells

2. 50µl of pre-diluted (1:2.5) inactivated positive control serum was added to all wells of col.1

3. 50µl was serially transferred by two-fold dilution from col.1 to col.2, and again from col. 2 col. 3 etc. until col.11. 50µl was discarded from col. 11

Mix plate I and II

1. 25µl was transferred from plate II to Plate I
2. 25µl of complement in 1:40 dilution was added to all cups of plate I
3. Plate I was added in a refrigerator, covered with second empty plate (cold fixation) or incubated in water bath at 37^0c for 30 minutes (hot fixation)
4. 50µl of 2% sheep red blood cells, amboceptor's premixture, equal volume, i.e.25µl of sheep red blood cells and 25µl of a 1:100 working dilution of amboçeptor's, was added to all cups
5. The plates were covered with sealing tape, shacked well and kept in water bath at 37^0c for 30 minutes (hot fixation)
6. The last cup with 50% sedimentation was read and recorded. The highest dilution of antigen with 50% sedimentation was the limiting antigen concentration or the right corner value. In this case the corner value was 1:25 dilution and was used through out the test. The 50% sedimentation was taken as one unit and the working dilution of the antigen was 2 units.

The test propre, multiple sera technique

1. The sera were prediluted to 1:2.5 and incubated at 58^0C in a water bath for 30 minutes in order to inactivate the native complement
2. 25 µl of diluted test sera was placed in wells of first and second rows of U-bottom plate, and 25 µl of veronal buffer was added to all wells except those of the first row
3. Serial doubling dilutions were then made by transferring 25 µl volume of serum from the second row onwards continuing for at least four dilutions
4. 25 µl of antigen diluted to working dilution excluding those of the anticomplementary controls, which received 25µl VBD instead
5. 25 µl of complement at working dilution (1.25) was added to all wells except control wells
6. Control wells containing: serum control has serum + complement + diluent and antigen control has antigen + complement + diluent. Complement control has complement + diluent and hemolytic system has diluent set up to contain 75 µl total volume in each case before hemolytic system was added
7. The plates were incubated at 37^0C for 30 minutes with agitations (warm fixation)
8. 25 µl of volume sensitized 2% SRBC suspension was added to each wells. The plates were sealed and re incubated at 37^0C for 30 minutes with agitations. Before reading the

result the plates were left in the refrigerator at $+4^0$C for 1 hour in order to allow none lysed cells to settle

9. Plates were taken out from refrigerator and left at room temperature for 10 minutes
10. Positive reactions were indicated by the absence of hemolysis, sedimentation of SRBC and negative reactions by the hemolysis of SRBC

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least with 50% fixation of complement (2+) at a dilution of 1:10 and at dilution of 1:20 were classified as positive (Alton *et al.*, 1975; OIE, 2000).

3.7 Data Analysis

Data from the laboratory results and questionnaires were stored in personal computer Micro soft Excel spreadsheet program. Descriptive statistical analysis of various risk factors and dependant variables were done using Intercooled STATA 7.0 for windows (stata corporation (2001) Texas USA). The chi square test or Fisher's exact test were used to test *Brucella* Seroprevalence rate and reproductive disorders association with incriminated categorical risk factors. Variables with substantial biological relevance were subjected to multiple logistic regression models to see whether there was a trend of association with the specified independent variable.

4 RESULTS

A cross sectional study was conducted in cattle and risky occupational groups from September 2003 to April 2004 in Sidama Zone, SNNPRG, Ethiopia. Serum samples (n=2438) were collected from cattle above six months of age. Out of these, 1627 (66.73%) were zebu cattle from all the six *Woredas*, where as 811 (33.26%) of them were Friesians and their crosses taken from two *Woredas*, namely Awassa and Yirgalem. The Friesian and their crosses were from intensively managed groups that made up 223 herds and zebus were from extensively managed 124 herds.

Thirty-eight human sera were also collected from occupationally exposed groups living in the study area. Besides, 223 dairy farmers engaged in urban and periurban dairying and occupational groups were also interviewed using a questionnaire format. Information regarding age, sex, breed and herd number was made available for all animals.

As there was no available health and reproductive performance record, the scope of the assessment was limited to major and easily remembered events of reproductive disorders in intensive management. These were abortion, stillbirth and retention of fetal membrane with or with out abortion or stillbirth. The occurrence and where possible, frequency of occurrence was recorded on herd level.

Table 1. Seroprevalence status of bovine brucellosis in Sidama Zone

Management systems	Number of animals sampled	Number of herds	No. and % positives (individual animals)	95%CI (individual prevalence)	No. and % positives for herds	95%CI (herd prevalence)
Intensive	811	223	20 (2.46)	(1.51-3.78)	15 (6.73)	(3.81-10.85)
Extensive	1627	124	27 (1.66)	(1.09-2.41)	17 (13.70)	(8.19-21.04)
Total	**2438**	**347**	**47 (1.92)**	**(1.42-2.56)**	**32 (9.22)**	**(6.39-12.77)**

4.1 Intensive management system (Urban and periurban system)

4.1.1 Individual level seroprevalence

Among 811 animals sampled from intensive management, 20 (2.46%) of them were found to react positive to screening and confirmatory test (Table 1). The individual level analysis of two *Woredas* were 2.67 % (n=411) for Yirgalem and 2.25% (n=400) for Awassa (Table 2).

4.1.1.1 Risk factors and brucellosis status in intensive management

Age

Categorization of age was made based on the biological relevance of the disease into three major categories. There was no positive reactor in the range 0.5 < 2 years, 2.89% reactors between 2 - 4 years and 3.03 % for the category greater than four years (Table 2). On pair wise comparison of the group's infection rate, the difference established between oldest and youngest group was not large enough to reveal significant difference. This was also true between 2 - 4 years and 0.5< 2 years.

Sex

In both *Woredas* (Awassa and Yirgalem), only 10 bulls were available for blood sampling and none of them were found to be positive for brucellosis. However, the prevalence in females was 2.49% (n=801) (Table 2). In this study, the number of male sample considered was not high to arrive at conclusion. Thus, comparing the two sexes is not justifiable from statistical point of view. Yet, the attempt was made to establish the likely association in order not to miss potentially important information.

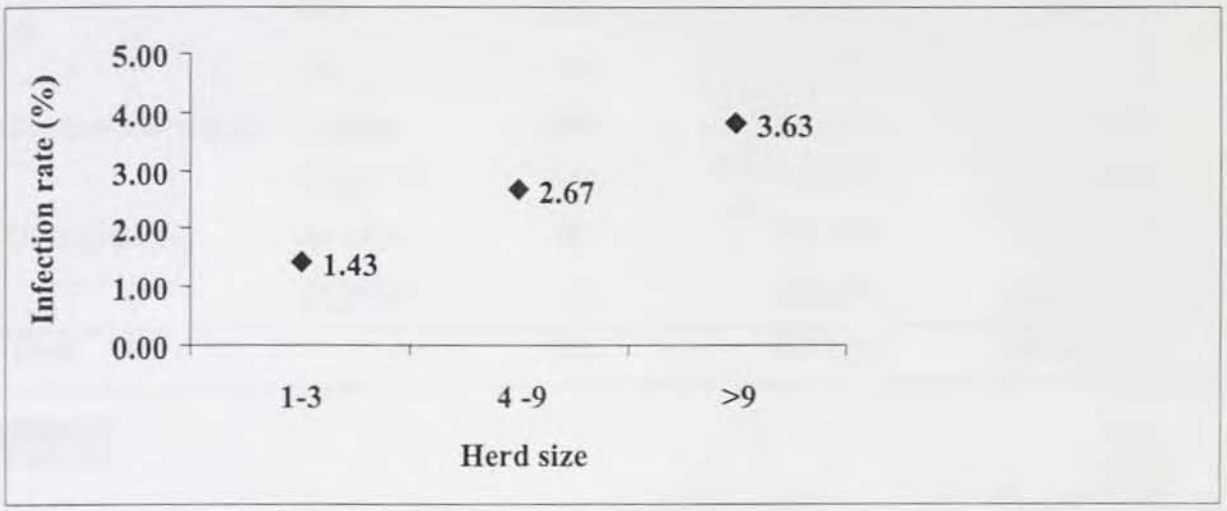
Herd size

In this study, the term herd was defined as the number of animals owned by an individual or family, which are kept together during night and day time. Since the average herd size observed for the two-management systems were different, grouping into small, medium and large was characterized differently. The bases for classification were type of labor, input, contribution to the family income, marketing and veterinary service.

Accordingly, small herd was characterized by family labor, minimum input (veterinary and feed reserve) and informal marketing of milk. Medium farms may or may not have hired

labor, largely dependent on return, feed reserve is available and market milk on contract basis to hotels or milk shops. Large farms use hired labor, own milk shop, keep some veterinary drugs in their stock and keep feed reserve at least for a quarter of a year. Furthermore, some hire professionals for regular visit and advice.

In intensive management, the highest prevalence of brucellosis (3.63%) was seen in a herd size above nine animals. The infection rate for the herd consisting 4 – 9 animals and farms that keep 1 to 3 animals were 2.67% and 1.43%, respectively (Fig. 1). Significant variation was not observed between large and small as well as between small and medium (P>0.05).



P>0.05

Fig. 1. Relationship of herd size and *Brucella* infection in intensive management

Table 2. Bovine brucellosis infection rate and attributed risk factors in intensive management.

Variables	Category	Sample size	No. &(%) positive reactors	95 % CI
Age	0.5<2	144	0	(0-2.52)
	2-4	173	5 (2.89)	(0.90-6.62)
	>4	494	15 (3.03)	(1.71-4.95)
Sex	Male	10	0	(0-30)
	Female	801	20 (2.49)	(1.53-3.82)
Herd size	1-3	272	4 (1.43)	(0.40-3.72)
	4 -9	374	10 (2.67)	(1.28-4.86)
	>9	165	6 (3.63)	(1.34-7.74)
Districts(individual)	Awassa	400	9 (2.25)	(1.03-4.22)
	Yirgalem	411	11(2.67)	(1.34-4.73)
District (Herd)	Awassa	98	5(5.10)	(3.90-14.22)
	Yirgalem	125	10(8.00)	(1.68-11.50)
Total		**811**	**20(2.45)**	**(1.51-3.78)**

P>0.05

4.1.2 Herd level (farm) seroprevalence

One or more reactors have been observed in 6.73 % (n=223) herds (Table 1). The herd level prevalence rates for Yirgalem and Awassa were 8 % (n=125) and 5.1% (n=98), respectively (Table 1). In this system, 12 of the herds had one positive reactor, one herd had two reactors and 2 herds were with three reactors each (Fig 2). The within herd prevalence were found to vary from 0 to 75 %.

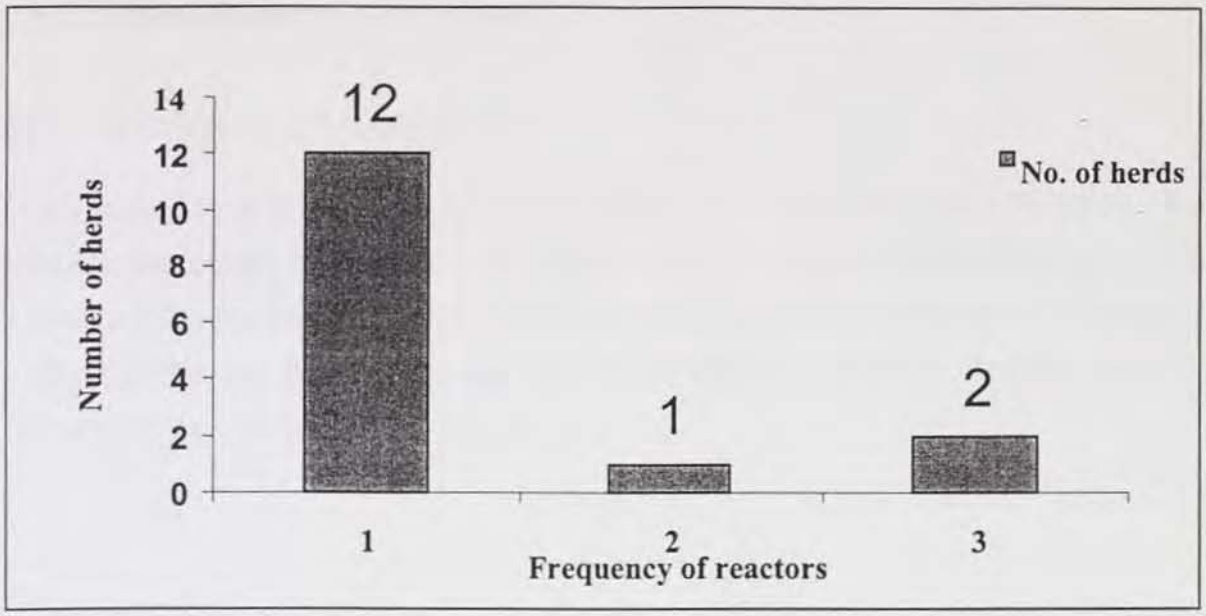


Fig. 2. Frequency of reactors recovered in positive herds (intensive management)

The scale used to analyze the infection status on the herd (farm) level was similar to individual level. The infection rates for the small, medium and large herds in intensive management system were 2.87% (n =139), 9.58% (n=73) and 36.36 % (n=11), respectively (Fig.3). The univariate logistic regression statistical analysis revealed significant difference between small and large ($P<0.01$) and also between medium and large herds ($P<0.01$). However, no significant difference was observed between medium and small.

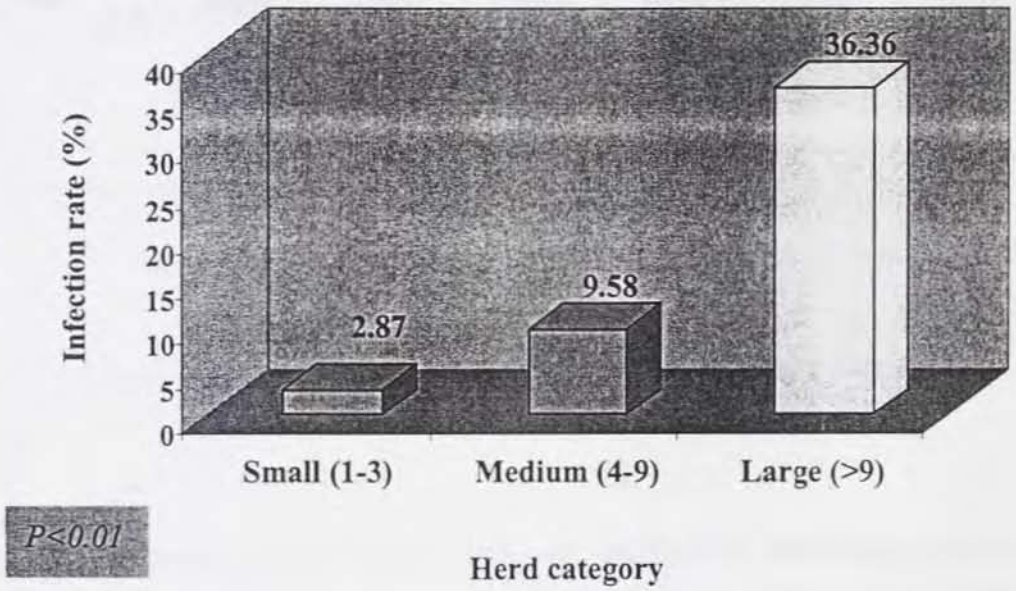


Fig. 3. Relationship of herd category and the herd level *Brucella* infection in intensive management system

4.2 Extensive management system

4.2.1 Individual level seroprevalence

In this management system, 1.66 % (n=1627) of the cattle were seropositive (Table 1). Their antibody titer ranged between 1:16 to 1:1024 (Annex 3). The brucellosis infection rate was 2.55% (n=274) for Awassa, 1.92% (n=313) for Yirgalem, 1.96% (n=254) for Aletawendo, 1.65% (n=242) for Hagereselam and 1.61 % (n=310) for Arbegona. Reactors were not recovered from Arroresa (n=234) (Fig. 4).

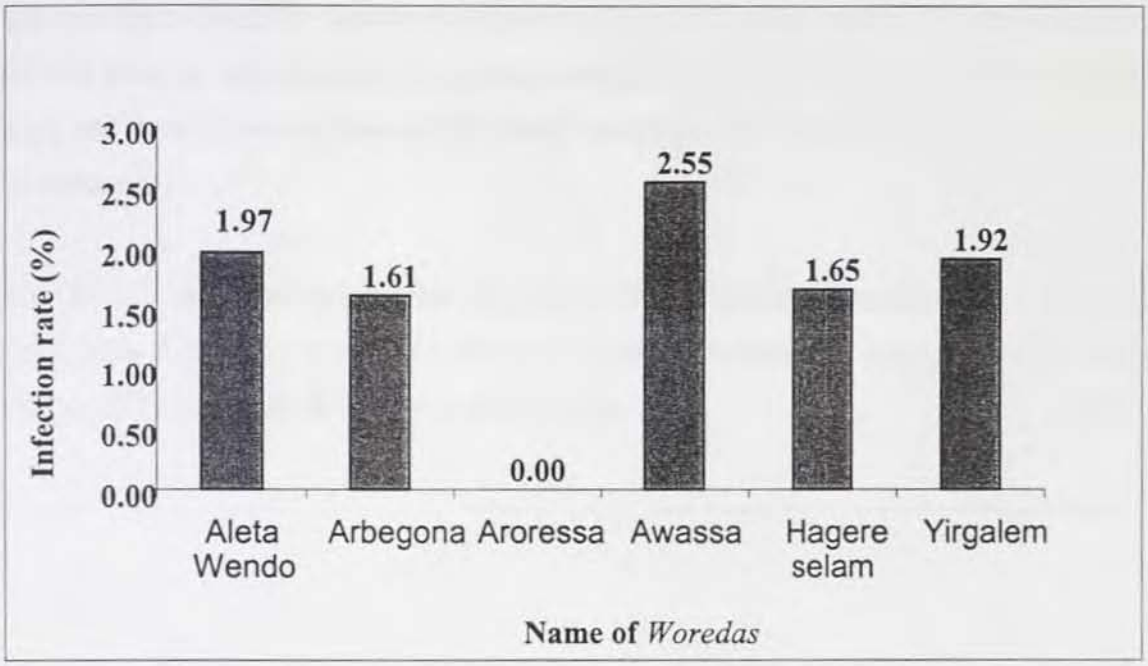


Fig. 4. Distribution of *Brucella* infection rate in extensive management

4.2.1.1 Risk factors and brucellosis status in extensive management

Age

Like the preceding management system, age stratification was made into 0.5<2 years, 2-4 years and >4 years. The seroprevalence rate was 0.49% for 0.5<2 years, 1.32% for 2-4 years and 1.96% for age group above 4 years (Table 3). The difference in infection rate among ranges was not significant (>0.05).

Sex

Out of the total of 1627 animals sampled, there were only 25 (1.5%) bulls in this study. Among these, one of them (4%) was positive. The seroreactor females were 1.62 % (n=1602). In this system also, most of the bulls were castrated as part of traditional breeding management and sampling of more number was not made.

Herd size

In the extensive management, small herd was characterized as the one kept as an additional asset; usually the family depends on coffee or other cash crop for livelihood and no feed reserve is kept by most. Medium herds are those groups of animals owned by a farmer who keeps crop residue or paddocks reserved for dry season. Farmers owning medium sized herd usually supplement their income from crops and livestock return. Sometimes, one of the family members takes the animals to the low land in search of forage. In large herd case, livelihood more or less depends on animals, owners give all their time and resource to their animals and usually two or more of the family members live with their cattle in the lowland (Transhumance).

In extensive management system, the large herd (>30animals) was found to have an infection rate of 2.06%. A prevalence rate of 2.37% and 1% were observed for medium (16-30animals) and the small (1-15 animals) herd, respectively (Fig. 5).

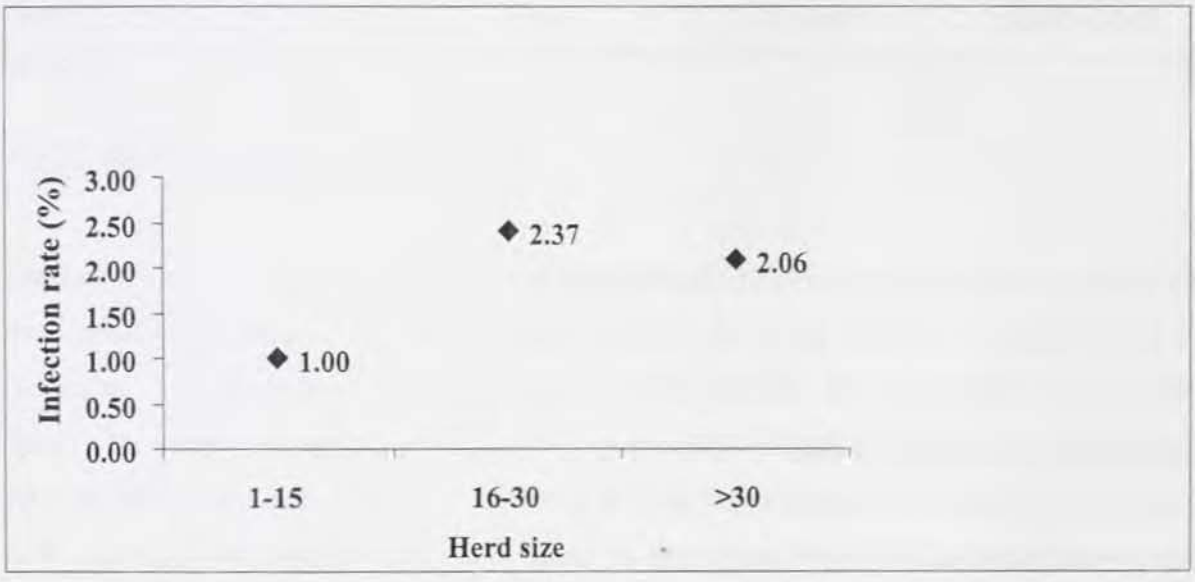


Fig. 5. *Brucella* infection rate versus herd size in extensive management

Table 3. Bovine brucellosis infection rate and attributed risk factors in extensive management.

Variable	Category	Sample size	No.& (%) positive reactors	95 % CI
Age	0.5<2	203	1 (0.49)	(0.01-2.71)
	2-4	302	4 (1.32)	(0.36-3.35)
	>4	1122	22 (1.96)	(1.23-2.95)
Sex	Male	25	1 (4.00)	(0.10-20.35)
	Female	1602	26 (1.62)	(1.06-2.36)
Herd size	1-15	696	7 (1.00)	(0.40-2.06)
	16-30	253	6 (2.37)	(0.87-5.09)
	>30	678	14 (2.06)	(1.13-3.44)
Districts	Awassa	274	7 (2.55)	(1.03-5.19)
	Yirgalem	313	6 (1.92)	(0.71-4.12)
	Aleta wendo	254	5 (1.96)	(0.64 -4.55)
	Hagere selam	242	4 (1.65)	(0.45 -4.17)
	Arbegona	310	5 (1.61)	(0.52 -3.72)
	Arroressa	234	0	(0-1.56)
Total		**1627**	**27 (1.66)**	**(1.09 -2.41)**

P>0.05

4.2.2 Herd (farm) level seroprevalence

Out of the 124 herds sampled in extensive management, 13.7% were found to be positive. The herd infection rates on *Woreda* basis were 33.33% (n=6) for Awassa, 15.38% (n=26) for Yirgalem, 13.15% (n=38) for Aletawendo, 14.29% (n=14) for Hagereselam and 18.18% (n=22) for Arbegona. In Arroresa (n=18) all herds were negative (Table 4). In this system, like the preceding once, a herd was defined as positive, if at least one animal is positive to both screening and confirmatory tests. Based on this, there was one herd with five reactors, four herds with two reactors, one herd with 3 reactors and 11 herds with one reactor (Fig . 6).

Table 4. Distribution of herd level infection in extensive management

Woreda	Herd size	No of positive herds	Inf. rate	95%CI
Awassa	6	2	33.33	(4.32-77.72)
Yirgalem	26	4	15.38	(4.35-34.86)
Aletawendo	38	5	13.15	(4.41-28.08)
Hagere selam	14	2	14.29	(1.77-42.81)
Arbegona	22	4	18.18	(5.18-40.28)
Arroresa	18	0	0	(0-18.53)
Total	**124**	**17**	**13.70**	**(8.19-21.04)**

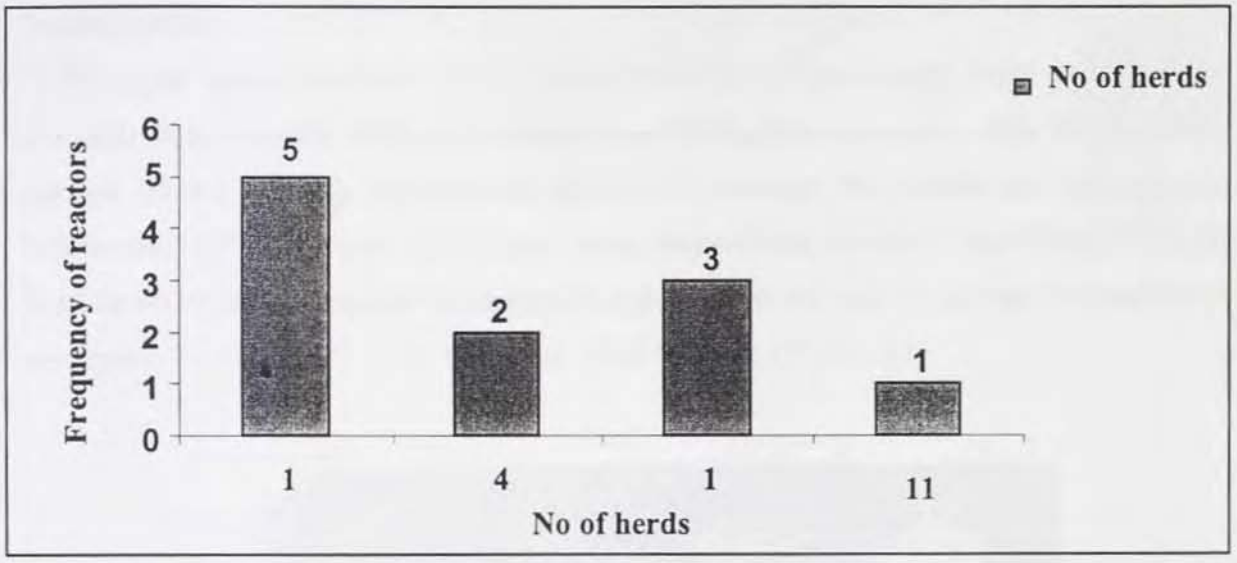


Fig. 6. Frequency of reactors in positive herds (extensive management)

With regard to the herd category, 4.81% (n=104), 50% (n=10) and 70% (n=10) were the infection rates for small, medium and large herds, respectively (Table 7). The variation here is apparently justified in univariate analysis between the small and large once ($P<0.01$) and between medium and small ($P<0.01$) but not between large and medium ($P>0.05$).

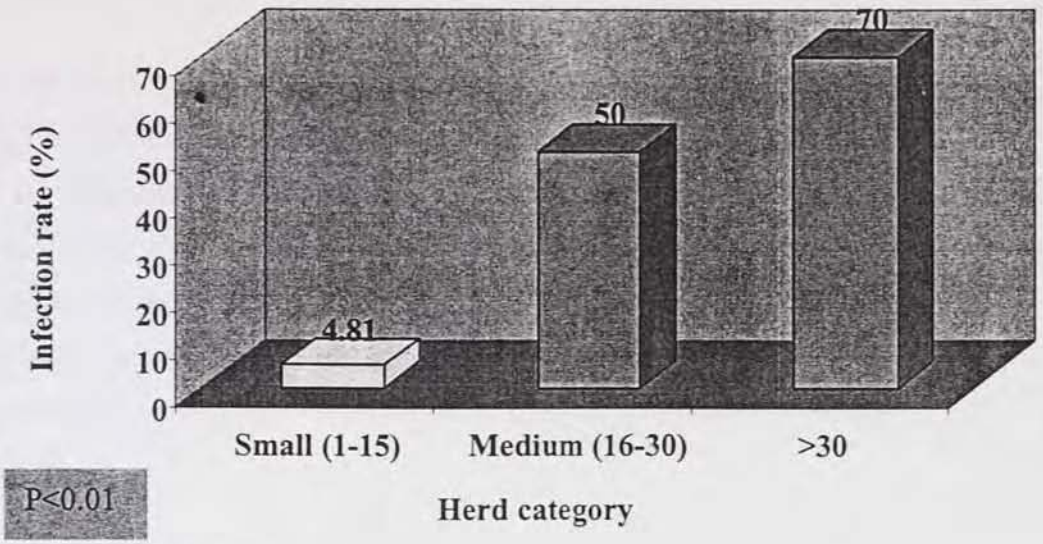


Fig. 7. Relationship of herd category with *Brucella* infection rate in extensive management at herd (farm)level

Pooled analysis

In this study, pooled analysis was only found possible to age category alone due to lack of similarity in parameters, category considered and biological explanation while characterizing the risk factors for both management systems. Accordingly the pooled age infection rate 0.29% and 2.20% for young and old age group, respectively, revealed significant difference in multivariate logistic regression analysis (i.e. between 0.5<2 and >2 groups) irrespective of management variation (P< 0.05, OR=1.85, 95%CI 1.08-3.19) (Fig. 8) .

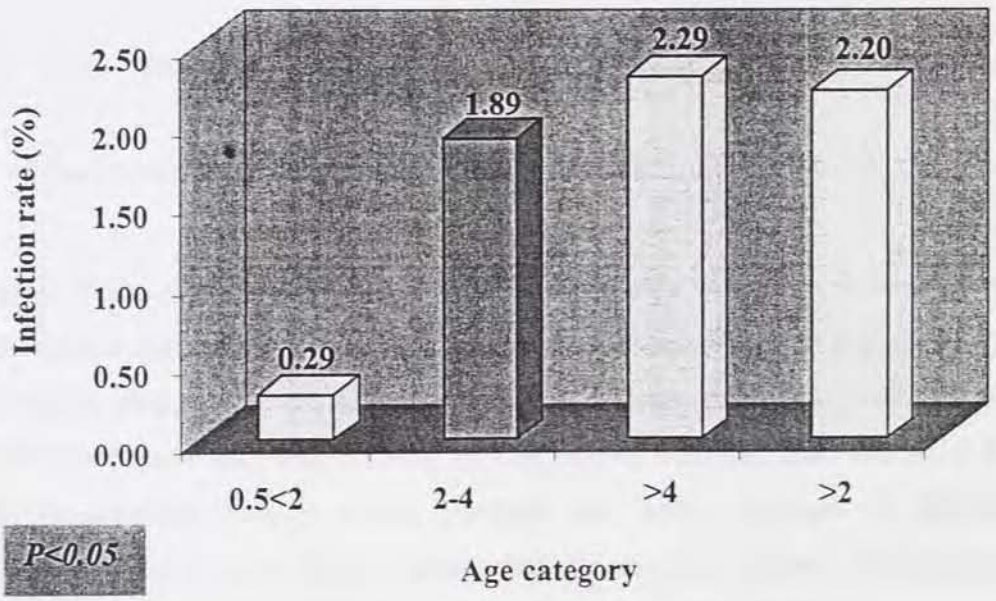


Fig. 8. Relationship of age and *Brucella* infection in extensive and intensive management

The presence of two different breed categories in this observation did not comfortably allow evaluating the difference to brucellosis infection, as they were kept entirely into two different management systems. The difference encountered rather ought to weigh the impact on husbandry practice than breed. In urban and periurban dairy system where the dominant composition was Friesians and their crosses, a prevalence rate of 2.46% has been appreciated. The picture for indigenous cattle in extensive management was found to be 1.66% (Fig.9). The infection rate in both cases was found to be uniform statistically (P>0.05).

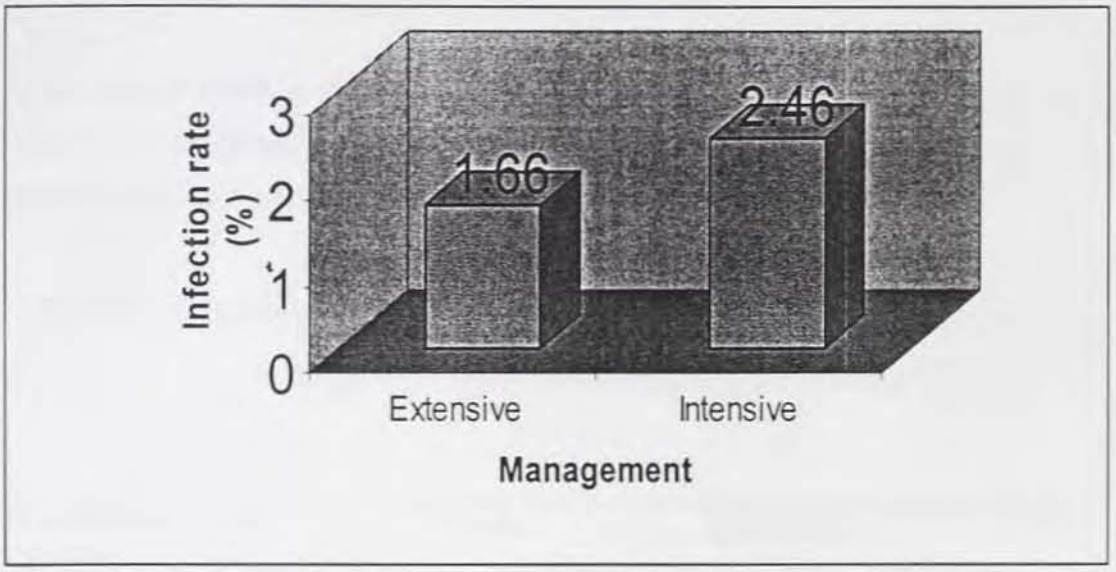


Fig. 9. Infection rate of bovine brucellosis in extensive and intensive management

4.3 Questionnaire result on management and husbandry

This aspect of the questionnaire evaluation on a herd basis was made to establish the role of extrinsic factors in the maintenance and transmission of brucellosis in intensive management. To this effect, data were collected on the presence of reproductive disorders (abortion, still birth and retention of fetal membranes), type of service used (AI, Bull and AI & Bull), herd contact, replacement strategy (raise, purchase and both), presence of parturition pen, separation of cows at parturition, cleaning and disinfection, method of placental disposal, housing, ventilation and drainage (Table 6) and culling reasons (Table 7).

4.3.1 Reproductive disorders and status of brucellosis

Since there was no farm with a record of complete health and reproductive parameters, it was hardly possible to evaluate all the reproductive performances. Therefore, the existing reality of the farms' management, limited the scope of the evaluation to major reproductive disorders that can be recalled easily on farm level. These were abortion, still birth and retained fetal membrane with or without abortion.

Reproductive disorders were reported from 17.49 % (n=223) herds. Among the herds that suffered any one of the specified disorders 28 animals were available for sampling. Of these 28 animals' sera, 32.14 % (9/28) were found to react positive for both RBPT and CFT. Sampling could not be made in the remaining 46.15% (18/39) herds, because animals that suffered reproductive disorders were culled.

In the survey result, a clear difference of brucellosis infection rate has been observed with large herd size (P<0.01, OR=4.46, 95%CI 1.85-10.45) and reproductive disorders (P<0.01, OR=8.9, 95%CI 2.95-26.81) (Table 5).

Table 5. Multivariate logistic regression analysis of brucellosis infection with large herd, reproductive disorder and improper fetal membrane disposal

Brucellosis infection	**OR**	**P value**	**OR 95%CI**
Reproductive disorders	8.9	< 0.01	(2.95-26.81)
Large herd (>9 animals)	4.46	<0.01	(1.85-10.45)
Improper fetal membrane disposal	3.59	P<0.05	(1.04-12.34)

Table 6. Descriptive statistics of the husbandry practice and reproductive disorders in intensive managements

	Number and percent of respondents					
	Yirgalem		Awassa		Over all	
Activities	**Number**	**(%)**	**Number**	**(%)**	**Number**	**(%)**
Natural mating	1	0.8	0	0	1	0.45
Use of AI	42	33.6	58	59.18	100	44.84
Both	82	65.56	40	40.81	122	54.71
Knowledge of brucellosis	3	2.4	14	14.28	17	7.62
Presence of parturition pen	0	0	2	2.04	2	0.89
Separation of cows during parturition	26	20.8	36	36.73	62	27.80
Cleaning and disinfection	0	0	2	2.04	2	0.89
Reproductive disorder	20	16.00	19	19.38	39	17.48
Herd contact	19	15.20	7	7.14	26	11.65
Improper placental disposal	65	52.00	39	39.79	104	46.63
Housing type stanchion	82	65.60	69	70.41	151	67.71
Loose housing	0	0	2	2.04	2	0.89
Cow shed	16	12.80	12	12.24	28	12.55
Living house	27	21.60	15	15.31	42	18.83
Ventilation and drainage						
Very good	0	0	2	2.04	2	0.89
Good	13	10.40	29	29.59	42	18.83
satisfactory	77	61.60	53	54.08	130	58.29
Poor	35	28.00	14	14.28	49	21.97
Replacement strategy						
raise own	11	8.80	13	13.26	24	10.76
purchase	0	0	1	1.02	1	0.45
both	114	91.20	84	85.72	198	88.87

4.3.2 Housing

Most of the farms in this study keep animals indoor. Especially in the urban farms due to lack of space, animals were only released or tethered in the compound during barren cleaning except occasional exercise. In the periurban areas, due to relative availability of space, animals were seen to have more opportunity to walk around.

The housing type encountered in urban or periurban area is categorized as "stanchion barn", loose housing, cow shed and living house type. These shelters of animals in both areas were largely traditional and hardly fulfill the criteria for stanchion housing type in the context. In some cases, cattle were found to share the main living house with the owners.

In Awassa, 70.41 % (69/98) of the houses and 65.60% (82/125) in Yirgalem were made of locally available materials and resemble one story stanchion barren. The roofing was made of corrugated iron, few with grass and walls with wood and mud. There were only two (2.04%), loose housing type at Awassa and none at Yirgalem. Cow shed account to 12.55% (28/223) of the housing types in the system.

The floor type is stone or concrete for 53.36% (119/223) of the houses and wood or timber for 16.59% (37/223) of houses in the settings. In both towns 30.04% (67/223) of houses have floor with compacted soil and stone. In Yirgalem and Awassa 21.60 % (27/125) and 14.28 % (14/98) of the farms, respectively, use rooms located inside the main living house. In this category both drainage and ventilation has been observed to be very poor. Nevertheless, with the other housing types, the ventilation and drainage was found to range from good to poor category at large (Table 6). There were only two modern stanchions barren observed at Awassa, which had very good ventilation and drainage system.

4.3.3 Culling reasons

According to the questionnaire result regarding culling, farmers cull their animals mainly for poor production and combination of reason.

Table 7. Culling reasons in urban and periurban dairy farms (n=223)

Variable	Number and percent of respondents		
	Awassa	**Yirgalem**	**Overall**
Old age	5 (5.1)	9 (7.2)	14(6.27)
Poor production	22 (22.4)	28 22.4)	50(22.42)
Reproductive disease	5 (5.1)	13 (10.4)	18(8.07)
Miscellaneous disease	7 (7.1)	6 (4.8)	13(5.82)
More than one culling reason	17(17.35)	23(18.40)	40(17.93)
No culling	31(31.63)	25(20.00)	56(25.11)
Others	11 (11.2)	21 (16.5)	32(14.34)
Total	**98**	**125**	**223(100)**

At Awassa, 17.35 % (17/98) of the respondents and 18.4 % (23/125) at Yirgalem claimed two or more reasons. In 31.63 % (31/98) of respondents at Awassa and 20 % (25/125) of respondents at Yirgalem culling has not been practiced yet (Table 7).

The reason given by those individuals who cull their animals exclusively for reproductive disease were repeat breeding, abortion, stillbirth, silent heat and uterine and vaginal prolapse.

The production loss or decline was largely attributed to mastitis and inferior traits. Low production was further aggravated by poor management such as feed shortage, chronic debilitating diseases, lack of labor, space, market, poor waste management and increasing feed price.

4.3.4 Breeding

In intensive management, 54.71% of the farms were found to use bull or AI depending on the availability and 44.84% the farms reported to use Artificial Insemination alone. Artificial insemination was generally the well-adopted service type in urban and periurban areas. In Awassa 59.18% of the farms and 33.6 % in Yirgalem use AI only and 40.81% of farms at Awassa and 65.56% of farms at Yirgalem have been using bull and AI depending on the availability. Only one farm at Yirgalem use natural mating alone (Table 6). Those farms, which use AI and Bull, claim AI being untrustworthy due to scarcity of consumables or lack of timely responding technicians.

4.4 Brucellosis in occupationally exposed groups

This part of the study was conducted in all *Woredas* of Sidama zone. In the zone, there are 57 animal health practitioners working in different districts (SZAD, 2003). Out of these, 66.7% (n=38) were available at the time of blood sampling and filled in the questionnaire. Among 38 individuals, 31.57% had one or more of the clinical manifestations of the disease and 5.3 % reacted positive to the screening and confirmatory test of CFT with the titer 1:64. The reactors have been in the profession for the last 18 to 20 years.

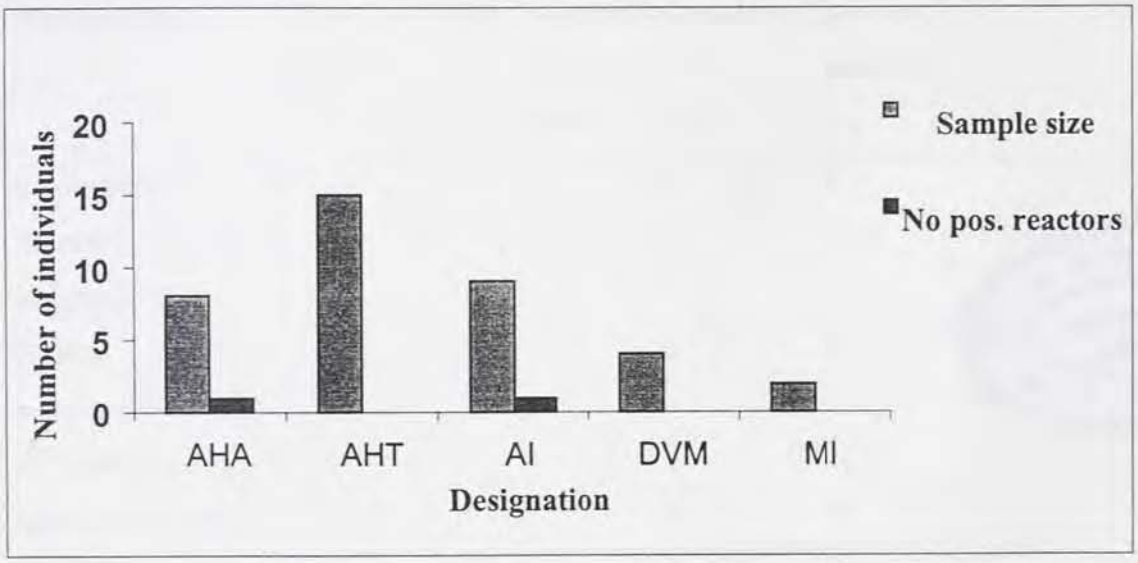


Fig. 10. Brucellosis seroreactors in risky occupational groups

Table 8. Results of questionnaires administered to investigate occurrence of brucellosis in man (n=38)

Variable	Number of respondents
Number of interviewee	38
Number of hospital visits in six month time	12
Reason of hospital visits for sick individuals (clinical complaints)	
Insomnia	0
Back pain	3
General malaise	10
Arthritis	2
Pain in the testes	1
Nervousness	0
Prolonged fever	7

However, none of the respondents was diagnosed for brucellosis by physicians, but for diseases like rheumatic arthritis, malaria, typhoid and unspecified febrile illness.

Table 9. Seroprevalence of *Brucella* antibody in animal health professionals

Designation of Post	No. of samples tested	Number of positive reactors		percent positive
		RBPT	CFT	
Veterinarians	4	0	0	0
Animal health assistants	8	1	1	12.5
Animal health technicians	15	0	0	0
AI technicians	8	1	1	12.5
Meat inspectors	2	0	0	0
Total	**38**	**2**	**2**	**5.3**

Individual level brucellosis infection rate

The study discloses that, the prevalence of bovine brucellosis in intensive and extensive management is found to be low. The low level infection rate found in this study was at variance with works of various investigators on intensive management involving Friesian and their crosses (Wondimu, 1989; Rashid, 1993; Sintaro, 1994; Asfaw *et al.*, 1998 and Bekele *et al.*, 2000).

Wondimu (1989) reported a prevalence rate of 15 % from central Ethiopia in crosses of Friesians. Rashid (1993) from IAR (Institute of Agricultural Research) and Sintaro (1994) from Chafa state farm reported 38.7% and 22 % infection rates respectively in intensively managed Friesian crosses. A prevalence rate of 8.11% in and around Addis Ababa was observed by Asfaw *et al.* (1998) and infection rate of 10.8% at Agarfa state farm was repoted by Bekele *et al.* (2000).

However, Belihu (2002), Tesfaye (2003) and Yayeh (2003) had similar findings in intensive management with this study. Belihu (2002) and Tesfaye (2003) could not find positive reactors in Selale area (n=747) and Mekele (n=100), respectively, in small dairy herds. Yayeh (2003), too, had observed an infection rate of 0.14 % (n=853) for Friesians and their crosses in small urban and periurban dairies of north Gondar. The possible explanation for this disparity could be the presence of large herd sizes in the former studies. For instance, Asfaw *et al.* (1998) and Bekele *et al.* (2000) had 50 animals as the upper limit of small herd category, 51-100 animals for medium and above 100 animals for large herds. Sintaro (1994) and Rashid (1993) were also having 182 and 147 animals in their respective herds. In this study, however there was only one herd with 24 animals and 95% of the herds kept under intensive management consists of less than10 animals. Furthermore, most of the owners keep their animals at home and the degree of contact between herds was very minimum.

Likewise, the infection rate observed in extensive management involving indigenous animals was also low. In this system, as well there are reports that agree with this finding. Shiferaw (1987) in Shoa, Wondimu (1989) in the central high lands of Ethiopia, Kebede (2000) in Eastern Amara National Regional State observed infection rates of 1.5%, 3% and 1.8%,

respectively, in local breeds kept under extensive management. The lower prevalence report of tropical high land (2%) (Omer *et al.*, 2000b) showed similarity among traditional management systems in this regard. In the area where sedentary livestock raising is predominant, the herd size and interaction among herds was limited to village level by most, contrary to pastoral area where mobility is the style. This might have limited the probability of the introduction of the disease.

Risk factors

Age

The majority of the reactors (97.87 %, n =47) were detected in age strata above 2 years in both management systems. This is a clear indication of age and sexual maturity being important determinants of the disease (Weidmann, 1991; Walker, 1999). However, in both management systems when age was treated separately, brucellosis infection has not demonstrated statistically justified trend of increment in parallel with age advancement. This could be due to smaller sample sizes for specified categories and lower overall prevalence. Pooling similar age categories together, considering mean age of sexual maturity (24 - 44 months) for zebu and crosses in Ethiopia (Mukassa- Mugurewa and Azage, 1991), clear difference was observed for age ($P<0.05$).

A relatively highest infection rate (2.29%) in older age group followed by in middle age (1.89%) and in younger age group (0.29%) was the trend observed in this study in pooled analysis. Despite the fact that the overall prevalence is low, a rise of infection rate has been observed in univariate logistic regression analysis (OR=1.85). This implies the probability of infection is nearly two fold in adults compared to the young group.

The rise of infection exhibited as age advances agree with reports of Bekele *et al.* (2000), Omer *et al.* (2000a), Asfaw *et al.* (1998) and Hellman *et al.* (1984). Bekele *et al.* (2000) could detect no reactor in animals less than 2 years. However, the prevalence rose from 1.7% in 2-4 years to 11.5% above 6 years, in Southeast Ethiopia. In and around Addis Ababa, Asfaw *et al.* (1998) had also reported 1.3% and 4% infection rate in the age range below 2 years and above four years, respectively. The recent 8.5% infection rate report of Omer *et al.*, (2000a) in Eritrea disclosed similar phenomenon in age between 2-4 years and no reactor

below. The highest infection rate in Dinka cattle (10.4%) and Felata cattle (26.2%) lie in the age range 4 to 8 years and the lowest rate in age less than two years (Hellman *et al.*, 1984).

Herd size

In both management systems, the established infection rate was found to increase together with the herd size from small to large. Nevertheless, the rate differences between herd categories were not significant statistically for either of the cases. In the situation where 95% of the herds in intensive and 83.3% of herds in extensive management comprise less than 10 and 15 animals, respectively, to analyze effect of herd size may be difficult. Omer *et al.* (2000a) reported 8.2% infection rate in individual animals in Asmara dairy farms; but as the majority (89.1%) of the farms he considered had less than 35 animals, no distinct trend was linked to herd size. Besides, the overall prevailing condition of the diseases status, which is 1.34% to 2.50% at 95% CI have less chance to illustrate the association of infection rate with herd size. The same phenomenon has been experienced in France, due to low level (0.07%) of *Brucella* infection in cattle, no powerful statistical analysis in infected herds could be performed to compare epidemiological pattern (Poullot *et al.*, 1998).

In this study much of the herds (70.59% in extensive and 80% in intensive) were considered positive on the presence of at least one reactor. In case of brucellosis, without any control measures, the intra herd rate of infections is more likely to increase with time, either with an exponential epizootic shape or with slow enzootic shape depending on the prevailing epidemiological situations (Nicolleti, 1980).

Sex

All the reactors in intensive management were females; no bull was found to react positive both to RBPT and CFT test. In the extensive management system, the infection rate for females was found to be lower (1.62%, n=1602) compared to male (4%, n=25) reactors. The finding here differ from the general agreement (Nicoletti 1980; Asfaw *et al.*, 1998). The presence of few bulls in dairy farms and wide castration practice in extensive management limited the number of male sample size. As the very purpose of dairying is milk and milk products, bulls were kept only in few farms. The tendency of keeping low number of bulls has also been observed in extensive management (Omer *et al.*, 2000a). The reason could be cost or/and additional husbandry practices in intensive dairy and traditional breeding practices in extensive system. In this study, bulls were considered not to miss important information. In

this regard, Lema *et al.* (2001) emphasized the high frequency of disease conditions in bulls that requires cautious consideration because of very small number of animals involved. Thus conclusion, regarding sex based on this result is difficult.

Management

The levels of *Brucella* infection rate tend to be lower in extensive management than intensive but this variation was not different statistically. On the contrary, there are several reports that prop up the rise of infection with change from extensive to intensive management whether these have indigenous cattle or introduced breeds (Omar *et al.*, 2000a, FAO, 1986). However, the finding here does not supporting this fact. Likewise, Bekele *et al.* (2000) reported seroprevalence of 2 % in extensive grazing and 3.4% in intensive dairy management system in southeast Ethiopia which agree with this finding.

Districts

The observation of reactors in all *Woredas* with the exception of Arroressa could be considered as evidence by itself for low but wide expansion of brucellosis in Sidama. The high herd level infection rate with *Woredas* reveals how much of the herds are involved in extensive management. This low (individual), high (herd) and wide (geographic) distribution is the cursor of likely threat of brucellosis in the future.

Brucellosis infection rate on herd level

Herd (farm) level infection rate is high in both intensive and extensive management system. The finding of higher infection rate in extensive than intensive looks contrary to well established facts, that brucellosis infection is more common in intensive than extensive management irrespective of breed difference (Omer *et al.*, 2000b). However, statistically the variation reported in this study is not substantiated. The difference is due to the variation of herd numbers sampled in respective management systems (i.e. 124 for extensive and 223 for intensive).

With the exception of age most categorical risk factors so far considered, had no significant statistical difference on individual level. Analysis made on herd level infection rate however, came up with a different scenario. To this effect, a positive herd was defined as any herd with at least one seropositive animal, and categorization of farms into small, medium and large is similar to individual analysis but the units here are herds not individual animals.

The association of infection rate in this study with the large, medium and small herds has statistical support ($P < 0.01$). In general, the herd level infection rate rise in parallel with the farm size increment was in agreement with reports of past studies. Asfaw *et al.* (1998) had reported infection rates of 17.6% and 100%, 100% for small, medium and large farms, respectively. The report of Bekele *et al.* (2000) in southeast Ethiopia was holding similar fact with 16.7%, 35.7% and 75% for small, medium and large farms, respectively. The situation in extensive management is similar, high infection rate for large followed by medium and small herds was observed. The difference observed between categories was strong enough to predict the hypothesized association of herd size to infection rate statistically.

This relationship is rationalized by the intense animal contact within the herd and especially following abortion (Nicoletti, 1980; Walker, 1999). Despite the variability what a large herd is, it is generally accepted that an increase in herd size is usually accompanied by increase in stoking size (Omer *et al.*, 2000a). Hellman *et al.* (1984), too, indicated that a high level of infection rate of bovine brucellosis was usually found in large herds compared to small herds.

The other important point observed and believed to compound the effect of intense contact in intensive management, is the poor hygienic standard (ventilation and drainage) of the farms in intensive management.

Reproductive disorders

The presence of high (17.48%) reproductive disorders (abortion, stillbirth and retained fetal membrane), reasonably explain the magnitude of the problem. The work of Lema *et al.* (2001) in urban and periurban dairy farms, has underlined the importance of reproductive diseases. In their cohort study around Addis Ababa, reproductive disorder was the second highest (11.5%) disease encountered next to mastitis.

In this study, a clear association of brucellosis infection with reproductive disorder has been observed ($P < 0.01$) at herd level in intensive management. Brucellosis is one of the reproductive diseases characterized by abortion, stillbirth and birth of unthrifty newborn (Walker, 1999; Dailey, 1992; Heino, 1989). The farm level brucellosis infection association with large herds ($P<0.01$) and improper fetal membrane disposal ($P<0.05$) was statistically significant. These observations show the importance of herd size in intensity of contact and

contamination of premises with discharge responsible to transmission of brucellosis (Radostits *et al.*, 1994).

Brucellosis in occupationally exposed groups

The overall seroprevalence (5.3 %, n=38) found in this study was suggestive of the *Brucella* exposure among surveyed groups. About one third of the individuals had one or more of the possible clinical manifestations, which lasted for more than five days in the last six months. All had hospital visit and yet, none was diagnosed for brucellosis. Perhaps this could be due to its high clinical manifestation resemblance to malaria, arthritis or hypochondriatic nature of patients (Madkar & Kasper, 2001). The detection of an antibody titer of 1:64 for both individuals might not show active infection but suggested exposure and inactive brucellosis or repeated exposure to antigenic stimuli (Araj and Azam, 1996).

It is a well-established fact that brucellosis among human population is largely influenced by the prevalence of the disease among domestic animals, through proximity of animals housing and processing of milk products (Alkalif *et al.*, 1992). In Egypt 67.9% of the human cases of brucellosis were associated with direct contact with sick animals (Taweel, 1999). Animal health professionals possibly contracted brucellosis while at work. In this regard Araj and Azam (1996) had stated that in the area where the infection rate was low, occupational exposure through direct contact with infected livestock or *Brucella* culture represent the major route of transmission.

6 CONCLUSIONS AND RECOMMENDATIONS

The sero epidemiological study has established a low infection rate of bovine brucellosis in both intensive and extensive management systems. The disease has been detected in all *Woredas* except Arroresa, from both intensive and extensive management systems. Due to this low level of infection rate, no risk factor could demonstrate distinct epidemiological association with brucellosis infection on individual basis apart from age.

The relative high herd level infection rate together with recovery of seroreactors in all *Woredas* except Arroresa, is a clear indication of its wide distribution. Brucellosis being an infectious and contagious disease, the time it needs to develop into major health and reproductive challenge will not be too far.

The detection of 5.3% seroreactors in professionally exposed groups is the evidence for the presence of occupational hazard even at low prevalence in animals.

The prevailing lack of awareness, poor hygienic status in intensive and herd contact at communal grazing land in extensive management are points to be stressed in view of control intervention.

In the light of the above conclusions, the following recommendations are forwarded.

Brucellosis must not be viewed as a disease of an individual animal, rather always in the context of the herd and the animal population of the region. In line with the current concept of "disease free zone" establishment in the country, brucellosis should be realized at least in urban and periurban dairy farms. To this effect, a law should be enforced to test all herds for brucellosis and accreditation should be given accordingly.

Effort should be made to create and develop awareness among smallholder dairy farmers to improve their over all husbandry practice including record keeping, housing and farm hygiene.

Intensive surveys and surveillances should be made for the better evaluation of brucellosis in the area.

Occupational groups at risk must abide the professional code of ethics and should work under safety precautions.

7 REFERENCES

Agab, H. (1997): Clinical signs of animal brucellosis in Eastern Sudan. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux* **50** (2), 97-98.

Allbala, S. R. (1995): Epidemiology of human brucellosis in southern Saudi Arabia. *Journal of Tropical Medicine and Hygiene* **98**, 185-189.

Akakpo, A. J., Bornel, P., Akayezu, J. M. V., Saradin, P. (1978): Epidemiology de la brucellose bovine en Afrique Tropicale,enquit sérologique,epidemiolgique et bactériologique au Rwanda. *Revue d' Elevag et de Medicine Veterinary des pays Tropieaux* **138** (3), 853-859.

Al-kalif, S. A. S., Mohamed, T. B. Nicoletti, P. (1992): Control of brucellosis in Kuwait by vaccination of cattle, sheep and goats with strain 19 or *Brucella melitensis* strain REV.1. *Tropical Animal Health and Production* **24**, 45-49.

Alton, G. G., Jones-Lois, M., Pietz, D. E. (1975): Laboratory technique in brucellosis. In: WHO monograph Series 55.World Health Organization, Geneva Switzerland, pp23-124.

Anthony, I. (2002): Urban dairing in Awassa, Ethiopia. Institute of Animal production in the Tropics and Sub-Tropics, University of Hoherheim, Stuttgart, Msc Thesis.

Araj, G. F. and Azzam, R. A. (1996): Seroprevalence of *Brucella* antibodies among persons in high risk occupation in Lebanon. *Epidemiology and infection* **117**, 281-288.

Asfaw, Y., Molla, B., Zessin, H. K., Tegene, A. (1998): The Epidemiology of bovine brucellosis in intra and periurban dairy production systems in and around Addis Ababa. *Bulletin of Animal health and production in Africa* **46**, 217-224.

Bekele, A., Molla, B., Asfaw, Y., Yigezu, L. (2000): Bovine brucellosis sero epidemiological study in selected farms and ranches in southeastern Ethiopia. *Bulletin of Animal Health and Production in Africa* **48**, 13-17.

Bekele, T. and Kassali, O.B. (1990): Brucellosis in sheep and goats in central Ethiopia. *Bulletin of Animal Health and Production in Africa* **38**, 23-25.

Bekele, T., Kassali, O. B., Mugurewa, M., Sholtens, R. G., Tamirat, Y. (1989): The prevalence of brucellosis in indigenous cattle in central Ethiopia. *Bulletin Animal Health and Production in Africa* **37**, 97-98.

Belhu, K. (2002): Analysis of dairy cattle breeding practices in selected areas of Ethiopia. Humboldt University, Berlin, PhD Thesis.

Bercovich, Z. and Muskens, J. A. M. (1996): The sensitizing effect of antigen in cattle after repeated intradermal inoculation. *Veterinary Microbiology* (an international journal) **51**, 85-93.

Chantel, J. and Bessiere, M. H. (1996): Serological survey of some zoonotic diseases among abattoir personnel in Djibouti. *Bulletin de la, S ociete de Pathologic Exotique* **89** (5), 353-357.

Chukwu, C. C. (1987): Brucellosis in Africa, Part II. *Bulletin of Animal Health and Production in Africa* **35** (2), 92-98.

Cooper, C. W. (1991): The epidemiology of human brucellosis in well defined urban population in Saudi Arabia. *Journal of Tropical Medicine and Hygiene* **94** , *416-422.*

Corbel, M J. (1997): Brucellosis an overview on emerging zoonoses. http://WWW.cdc. gov/ncidod/EID/ vol3no2/downcorb.h.

Central Agricultural Census Comission (2003): Statistical report on farm management practices, livestock and farm implements part II. Addis Ababa, July, 2003.

Currier, W. R. (1989): Zoonosis update. *Journal of American Veterinary Association* **195** (5), 595-597.

Dailey, R. A. (2001): Abortion in dairy cows and heifers, north east Virginia. IRM manual, http://WWW.inform.umd.edu./EdRes/topic/Ag/Abortion in dairy cows and heifers. html.

El-Ansary, E. H., Mohamed, B. A., Hamad, A. R. A., Karom, A. G. O. (2001): Brucellosis among animals and humans contacts in Eastern Sudan. *Saudi Medical Journal* **22** (7), 577-579.

FAO/WHO (1986): Joint FAO / WHO expert committee on brucellosis six reports. Geneva: WHO.740 (WHO technical report series; 740).

Garin-Bastuji, B. and Verger, J. M. (1994): *Brucella abortus* and *Brucella melitensis.* In: International Dairy Federation (ed.): The significance of pathogenic microorganisms in milk. Paper presented at international workshop held in Brussels, Belgium, 6-11th Feb, 1994. pp 168-185.

Hamdy, M. E. R. and Amin, A. S. (2002): Detection of *Brucella s*pecies in the milk of infected cattle, sheep, goats and camels by PCR. *The Veterinary Journal* **163,** 299-305.

Heino, M. (1989): Artificial Insemination of cattle in Ethiopia. Ministry of Agriculture, Addis Ababa, Ethiopia, PP 71-94.

Hellman, E., Staak, C., Bauman, M. (1984): Bovine brucellosis among two different cattle populations in Baher el ghazal province of Southern Sudan. *Tropical Medical Parasitology* **35**, 123-126.

Hussen, A. S., Singh, S. S., Hajji, H. (1978): A survey of bovine brucellosis in southern part of Somalia Democratic Republic. *Bulletin of Animal Health and Production in Africa* **26** (3), 150-153.

Jiwa, S. F. H., Łazawala, R. R., Tungaraza, R., Kimera, S. I., Kalaye, W. J. (1996): Bovine brucellosis agglutination test prevalence and breed disposition according to prevalent management systems in Lake Victoria zone of Tanzania. *Preventive Veterinary Medicine* **26**, 341-346.

Kabagambe, E. K., Elzer, P. H., Geagen, J. P., Opuda-asibo, J., School, D. T., Miler, J. E. (2001): Risk factors for *Brucella* seroposetivity in goat herds in Eastern and Western Uganda. P*reventive Veterinary Medicine* **52,** 91-108.

Kagumba, M. and Nandokha, E. (1978): A survey of the prevalence of bovine brucellosis in East Africa. *Bulletin of Animal Health and Production in Africa* **26** (3), 224-229.

Kebede, F. (2000): An Epidemiological survey of bovine brucellosis in Amara National Regional State. Proceedings of the 14th annual Ethiopian veterinary Association. Conference held at Addis Ababa, 7th-10th June 2000.

Konrad, W. (1981): Brucella. In: Felik, M., John, A. C., Kanoo, K. (eds.): Principles of Immunological Diagnosis in Medicine. Philadelphia: Lea & Berger. pp 97-101.

Lema, M., Kassa, T., Tegene, A. (2001): Clinically manifested major health problems of crossbred dairy herd in urban and periurban production system in the central high land of Ethiopia. *Tropical Animal Health and Production* **33**, 85-93.

Madkar, M. M. and Kasper, L. D. (2001): Brucellosis. In: Branwald, E., Housen, S., Fauce, A., Kasper, L. D., Longo, D., Jamson, L. J. (eds): Harrison's Principles of Internal Medicine, 15th ed. Vol.1. International edition. McGraw Hill companies, PP 986-989.

Madson, M. (1986): The current state of brucellosis in Zimbabwe. *Zimbabwe Veterinary Journal* **20** (4), 133-141.

Mahajan, N. K. and Kulshreshtha, C. R. (1991): Comparison of serological tests for *Brucella melitensis* infection in sheep. *Tropical Animal Health and Production* **23,** 11-16.

Maiga, S., Traore, M. D., Niang, M., Toure, I. 1996): Seroepidemiological investigation of bovine brucellosis in the dairying belt of Bamako, Mali. Proceedings of 18th International conference held at Bamako January 1996. pp 289-292.

Mc Dermot S., John, J., Arimi, S. M. (2002): Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology* **90,** 111-134.

Mekonnen, G. (2001): Seroepidemiological investigation of bovine brucellosis in north eastern Ethiopia (unpublished, Baherdar Regional Laboratory report).

MOA (1998): The role of village dairy cooperatives in dairy Development: Prospect for improving dairy in Ethiopia. Papers presented at SDDP work-shop held in Addis Ababa, Ethiopia, 22-24 th April, 1998. pp4 -7.

Molla, B. (1989): Seroepidemiological survey of bovine brucellosis in Arsi region. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM Thesis.

Mukassa, M. and Tegene, A. (1991): Reproductive performances in Ethiopian zebu (*Bos indicus)* cattle constrain and impact on production. In: Institute of Agriculture (ed.): Paper presented at a Workshop of the Fourth National Livestock Improvement Conference, Addis Ababa, Ethiopia, 13-15, November 1991. pp 16-28.

Mustefa, M. and Nicoletti, P. (1993): FAO, WHO, OIE guidelines for regional brucellosis control programme for the Middle East. Prepared at a workshop held in Amman, Jordan, 14 -17 February, 1993.

Nicoletti, p. (1980): The epidemiology of bovine brucellosis. In: Brandly, C. A., Corneleius, G.A. (eds): Advance in Veterinary Science and Comparative Medicine. New York Academic Press Inc., Pp 68 – 69.

Nielsson, K. H., Kelly, L., Gall, D., Balcevicus, S., Bosse, J., Nicolleti, P., Kelly, W. (1996): Comparison of enzyme immunoassays for the diagnosis of bovine brucellosis. *Preventive Veterinary Medicine* **26,** 17-32.

OIE (1996): Bovine brucellosis. Manual of standards for diagnostic tests and vaccine. Paris: Office International Des Epizootics. pp 242-262.

OIE (2002): Bovine brucellosis. Manual of standards for diagnostic tests and vaccine. Paris: Office International Des Epizooties. pp 328-345.

Ollofs, A., Baumann, M. P. O., Afema, J., Nakavuma, J. (1998): Experience with the strategy to investigate bovine brucellosis in rural areas of south West Uganda. *Revue, d'Eeevag et de MedicineVveterinary des pays Tropecaux* **51** (2), 101 – 105.

Omer, M. K., Assefaw, T., Skjerve, E., Holstd, G., Woldehiwot, Z. (2000a): Risk factor for *Brucella* species. Infection in dairy cattle farms in Asmara, state of Eritrea. *Preventive Veterinary Medicine* **46,** 257-265.

Omer, M. K., Skejerve, E., Holstd, G., Woldehiwot, Z., Mackmilan, A. P. (2000b): Prevalence of antibodies to *Brucella* species in cattle, sheep, goats, horses and camels in the state of Eritrea, influence of husbandry system. *Epidemiology and Infection* **125**, 447-453.

Omer, M. K., Assfaw, T., Skjerve, E., Teklegiorgis, T., Woldehiwot, Z. (2002): Prevalence of antibodies to *Brucella* species and risk factors related to high risk occupational groups in Eritrea. *Epidemiology and Infection* **129**, 85-91.

Peter, T. A. (2000): Abortion in dairy cows: new in sight and economic impact. http :// WWW.afns.ualberta.ca/hosted/wcds/wcd2000/proceedings/chapter19.htm.

Quinn, P. J., Carter, E. M., Markey, B., Carter, R. G. (1994): *Brucella* species. In: Clinical Veterinary Microbiology. Mosby international limited. PP 261-267.

Rana, U. V. S., Shegal, S., Mohan, B. (1985): A seroepidemiological study of brucellosis among workers of veterinary hospitals and slaughterhouse of union territory of Delhi. *International Journal of Zoonoses*, **12,** 74 -79.

Radostits, O. M., Blood, D. C., Gay, C. C. (1994): Brucellosis caused by and *Brucella melitensis.* In: Veterinary Medicine, textbook of the disease of cattle, sheep, pigs, goats and horses, 8th ed. London: Bailliere Tindal. pp 787-803.

Radostits, O. M., Lesilie, E. K., Fetrow, J. (1994): Diagnosis of bovine infectious abortion disease. In: Heard health: Food animal production, Medicine, 2nd ed. W.B. Soundress Company. pp 269-272.

Rashid, M. (1993): Reproductive wastage in cattle due to bovine brucellosis. Institute of Agricultural Research (ed): Proceedings of the Fourth National Livestock Improvement Conference, held at Addis Ababa, 13-15th November,1993, pp 270-272.

Redkar, R., Rose, S., Bricker, B., Delvachio, V. (2001): Real time detection of , *Brucella melitensis* and *Brucella suis*. *Molecular and Cellular Probes* **15,** 43-52.

Russel, W. C. (1989): Zoonosis update, brucellosis. *Journal of American Veterinary Medical Association* **195** (5), 595-597.

Schuring, G. G. (1998): vaccine: smooth and rough strains. OIE scientific and technical review, **17**(1). pp 200-219.

Schelling, E., Diguimbaye, C., Daoud, S., Nicolleti, J., Boertin, P., Tanner, M., Zinnstag, J. (2003): Brucellosis and Q. fever seroprevalence of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine* **61**, 279-293.

Seifert, H. S. (1996): Brucellosis, In: Tropical Animal Health, 2nd ed. Dordrecht: Kluwer Academic Publishers Group, PP 358-362.

Shiferaw, A. (1987): The prevalence of bovine brucellosis under different management system around Shoa. Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, DVM Thesis.

Sidama Zonal Agricultural Department (2003): Personal communication.

Sidama Zone Planning and Economic Development Department (2001).

Sintaro, T. (1994): The impact of brucellosis on productivity in an improved dairy herd of Chafa state farm, Ethiopia. Fachbeich Veterinaemedizin, Freie Universitaet Berlin, Berlin, Msc Thesis.

Smith, C. M. and Sherman, M. D. (1994): Brucellosis. In: Carrol, C. C .and Hungerbergen, S. (eds.): Goat Medicine. Baltimore: Lea and Febiger, pp 423-424.

Staak, C. (1990): Serological techniques in brucellosis and interpretation of results. First international conference on brucellosis, 19 – 20th March, 1991. .Mosul, Iraq.

Stata Corporetion (2001): Intercooled Stata 7.0 for Windows 98/95 NT. 702 University Derive East College Station, Texas, USA.

Sutra, L., Caffin, J. P., Dubray, G. (1986): Role of milk immunoglobulin in the *Brucella* milk ring test. *Veterinary Microbiology* **12**, 59-366.

Taweel, A. E. (1999): The actual situation and legislation aspects of brucellosis in Egypt. http: // WWW.More-in.org /EG/ bruc-taweel.html.

Thrusfield, M. (1995): Sampling. In: Veterinary Epidemiology, 2nd ed. London: Black Well Science Ltd., PP 179-284.

Tizard, I. (1996): Vaccination and vaccines. In: Veterinary Immunology, an Introduction, 5th ed. Philadelphia: W.B. Saunderes Company, PP 265-283.

Verma, S., Ramesh, C. K., Sharma, M., Nigam, P. (2000): Abortion and infertility in domestic livestock due to brucellosis in Himachel pradesh, India. *Vetrenariski Archiv* **70**, 75-82.

Walker, R.L. (1999): *Brucella*. In: Dwight,C.H. and Chunge Z.Y. (eds): Veterinary Microbiology. Massachusetts, Black Wells Science, pp196-203.

Weidmann, H. (1991): Survey of means now available for combating brucellosis in cattle production in the tropics. In: Animal research and Development.Vol.33. Tuebingen Institute for Scientific Cooperation, pp 100-111.

Wernery, U., Kerani, A. A., Viertel, P. (1979): Bovine brucellosis in Southern Somalia Democratic Republic .*Tropical Animal Health and Production* **11**,31-35.

WHO (1997): Emerging and other communicable disease surveillance and control. The development of new/improved brucellosis vaccines. Reports of the WHO meetings, Geneva, December, 1997. pp 1 – 47.

Wondimu, A. (1988): The epidemiology and economics of bovine brucellosis in the central highlands of Ethiopia. Veru: Reading University, Msc Thesis.

Zewdu, E. (1989): Seroprevalence study of bovine brucellosis in selected sites of Sidamo region. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM Thesis.

8 ANNEXES

Annex 1. Questionnaire format for intensive dairy farms.

I- General information Date --------

Name of the owner_____ Sex_____ Age_____yrs

Occupation_____

Address_____

Year dairy farming started_____.

Are you practicing dairy as the only way of life? Yes____ No____

Location Urban_____ Peri urban_____

Labor? family___ Hired_____

Market? Milk shop____ Hotel_____ Informal_____

Feed reserve (at least for 4 months) Yes_____ No_____

Veterinary service on call basis_____ Regular basis_____

II-Information on the herd.

Herd size _____

Is there bull in the farm? Yes___ No_____

Is there frequent contact between your animals with other herds? Yes___ No____

Breed- Local_____ Friesian_____ Cross_____ Others_____

No of milking cows_____No of first lactation heifers_____ No of female calves_____.

III-Information on Brucellosis

What are your culling criteria? Reproductive problems___ Non-reproductive disease_____

If reproductive problems what are they? _____

_____

_____

____

Old age____ Poor production_____ other (specify) _____

What type of service do you have for your animals?

AI____Bull____ Both___

Do you know brucellosis? Yes___ No____

Are there separate parturetion maternity pen (s)? Yes___ No____

Do you separate cows during parturition? Yes__-No___

What do you do to the calving pen after parturition? _____

_____

_____

How do you dispose off the after birth?

_____

Where do you get the replacement stock? Buy in_____ raise own replacement_____

Both_____

What do you do with the known *Brucella* infected animals?

_____

_____

Have you observed abortion/ stillbirth in your farm? Yes____ No____

How many abortions/stillbirths or retained after birth have you encountered during the last three years? _____

No of abortion____ . No. of still birth ___. No. of retained fetal membrane _____

If the animals are there,

Sample no of abortion_____, still birth___ and retained after birth___

When did the abortion occur? 1st trimester____ 2nd trimester____ 3rd trimester____

Did the farm been tested for brucellosis? Yes___ No____

If yes when_____

Did vaccination for brucellosis carried out since inception? Yes___ No___

If yes when_____

IV-Farm Setting up report

Housing

Building material: traditional_____

modern_____

combination_____

Housing type Stanchion baren____

Loose housing_____

Cow shed_____

Other_____

Floor type Earth____

Concrete_____

Wood_____

Stone_____

Other____

Does the building allow sunshine into the house? Yes____ No_____

If parturition pen is there, is it clean and dry? Yes____ No____

Are dry cow facilities clean? Yes____ No____

Are cow stalls of adequate size (220cm×110cm) Yes____ No____

Drainage and ventilation.

Very good_____

(Sufficient open space below roofing frames no ammonia odder, raised roofing, clean and dry, and elevated bedding space, non- absorbable floor with drainage grove.)

Good_____

(Clean and dry, open space at one side of the wall, non-absorbable and inclined floor space)

Satisfactory____

(Clean and dry, no sufficient opening for ventilation).

Poor_____

Dirty& wet floor space, no sufficient drainage and ventilation.

General farm hygiene and standard

Very good_____ Good_____ Satisfactory_____ Poor____

Annex 2. Format to investigate the occurrence of brucellosis in risky occupational groups.

Occupation Vet ---- AHA------ AHT------ AI T------ Meat inspector---------

Service year _____

What precaution do you take in handling retained after birth management?

Have you visited health institution during the last 6 months? Yes---- No----

Did you have any of the following clinical signs and symptoms lasted for more than 5 days during the past six-months?

Headache yes--- No----

Insomnia Yes--- No----

Pain over spine Yes--- No----

General malaise Yes--- No---

Joint pain yes ----- No-----

Pain in testes Yes-----No ------

Nervous disorders yes----- No------

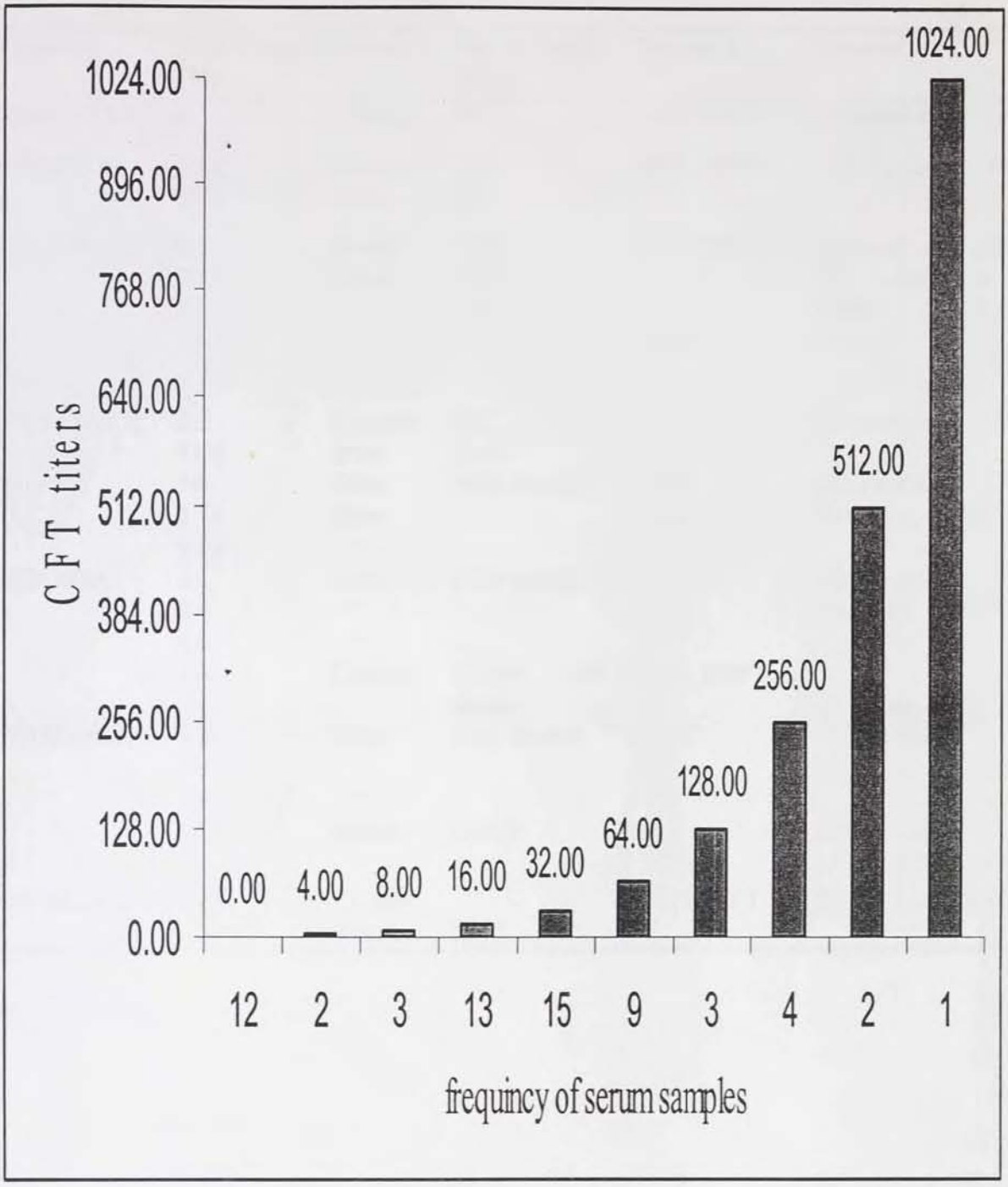
Vet. Veterinarian

AHA Animal Health Assistants

AHT Animal Health Technicians

AI Artificial Insemination Technicians

Annex 3. Distribution of antibody titers in 64 RBPT positive sera



Annex 4. Prevalence of bovine brucellosis in East Africa

Country	Prevalence (%)	Breed	No. of cattle tested	Test used	Source
DJIBOUTI	4	Zebu	499	SAT, CFT	(Chantel *et al.*, 1994)
RWANDA	35.1	Ankole	510	RBPT, CFT	(Akakpo *e t al.*,1994)
	33.1	Mitis	141	" "	"
SUDAN	6.5	Dinka	5982	SAT, CFT	(Helman *et al.*,1984)
	22.5	Felata	1228	" "	(El Ansary *et al.*,
	5	NS	1225	TAT	1999) (Agab.H,
	13.3	NS	916	RBPT	1996)
SOMALIA	2.5	Crosses	902	SAT	(Hussen *et al.*, 1978)
	11.9	Zebu	2184	"	"
KENYA	10	Zebu	1036 (total)	RBPT	(Kagumba and
	3.76	Zebu		SAT	Nandoka, 1978)
	9.02	"		CFT	"
UGANDA	5	Zebu	1739 (total)	RBPT	(Kagumba &
	4	"		SAT	Nandoka, 1978)
	4.6	"		CFT	"
	1.8	Crosses	13599 (83 herds)	MRT, RBPT CFT	" (Olofs ,1998)
TANZANIA	5.8	Zebu	23017(total)	RBPT	(Kagumba and
	4.8	"		SAT	Nandoka , 1978)
	5.0	"		CFT	"
	10.8	Mixed	13078	SAT	(Jiwa *et al.*, 1996)
ERITREA	8.2	Crosses Friesian	2427	RBPT, CFT	(Omer *et al.*, 2000a)

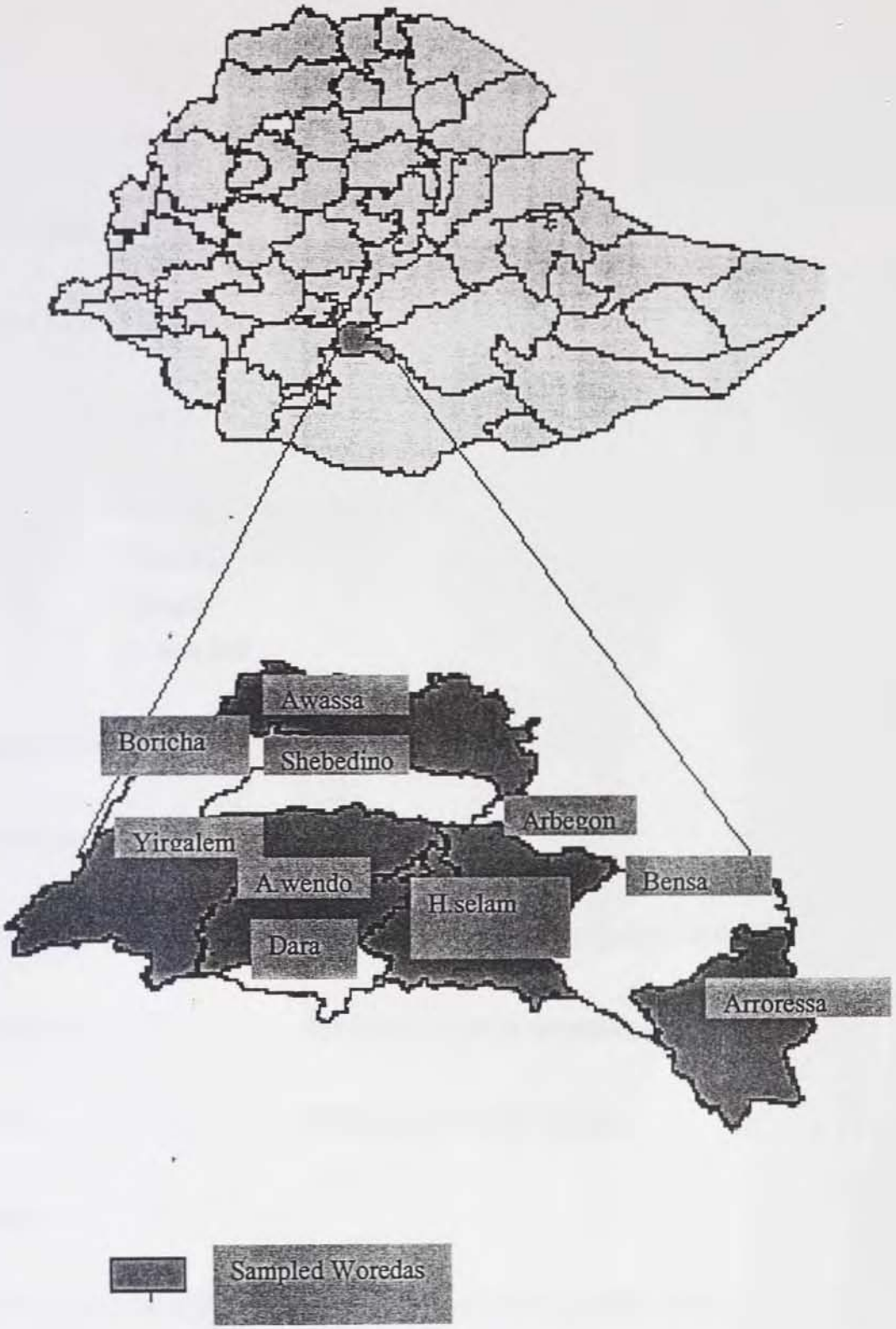
Ns = *Not specified*

Annex 5. Prevalence of bovine brucellosis in Ethiopia

Breed	Prevalence %	No examined	Prevalence %	Test	Area	Source
Zebu	4.2	1606	4.2	RBPT	Ghibe	(Bekele *et al.* ,1989)
Mixed	8.86	2178(Total)	8.86	RBPT	Arsi	(Molla, 1989)
"	7.62		7.62	SAT		"
Crosses	15	NA	15	RBPT&SAT	Central	(Wondimu,
Zebu	3	NA	3	"	Ethiopia	1989)
						"
Crosses	15.8	734 (5 herds)	15.8	RBPT	Sidamo	(Zewdu,
"	11.6		11.6	SAT		1989)
Crosses	38.7	147	38.7	RBPT, SAT	IAR farms	(Rashid, 1993)
Crosses	22	182 (1 herd)	22	RBPT, SAT	Chafa	(Sintaro, 1994)
Friesian & Crosses	8.11	1114	8.11	MRT, RBPT, CFT	In and around Addis Ababa (urban&periurban)	(Asfaw *et al.*, 1998)
Mixed	19.4	4094 (total)	19.4	MRT, RBPT	Abernossa,	(Bekele *et*
Friesian	10.8		10.8	,CFT, ELISA	Agarfa and	*al.*, 2000)
mixed	6.3		6.3		Didatiura ranches	
Zebu	1.8	3644	1.8	RBPT&CFT	ANRS (eastern part)	(Kebede, 2000)
Mixed	8.3	NA	8.3	RBPT	ANRS(western part)	(Mekonen, 2001)

NA = not available

Annex 6. Map showing the study Area (Sidama Zone)



9 CURRICULUM VITTE

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Sex Male

Date of Birth Nov.1968GC

Place of birth Yirgalem

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Address SNNPRG, Sidama zone
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Ethiopia
po. box 219

Language (Spoken and Written) Amharic and English

Educational background

1-Elementary and junior (1-8) Oddo Shakisso elementary and junior School

2-Highschool (9-10) Kibremengist senior secondary high School

3-High school (11-12) Yirgalem C/S/S/High School

Colleges

1-Addis Ababa University, Faculty of veterinary medicine (1991-1996)

Degree awarded DVM

2- Addis Ababa University, Faculty of veterinary medicine (2003-2004)

Degree awarded Msc in Tropical Veterinary Epidemiology

Work experience

1-Distict vet. Section team leader

2-Trainer of AI technique

Research

1-Antihelmintic Sensitivity of GIT Nematodes in Small Ruminants in SNNPRG

2-Preliminary study on Ethenovetrinary medicine practice in Sidama

3-Epidemiology of brucellosis in cattle and its Seroprevalence in Animal Health Professionals in Sidama Zone (Southern Ethiopia)

10 SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any university.

Name Kassahun Asmare

Signature---Dassah A.---

Date of submission ---14th June 2004

This thesis has been submitted for examination with our approval as University advisors.

Epidemiology of brucellosis in catile & its sero prevelence in animal health professionals in Sidama Zone, Southern Ethiopia

Kassahun Asmare